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DIFFERENTIAL $[C^{14}]$ 2-DEOXYGLUCOSE UPTAKE AFTER DEAFFERENTATION OF THE MAMMALIAN AUDITORY PATHWAY - A MODEL FOR EXAMINING TINNITUS

Lisa Babitz

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DIFFERENTIAL $[C^{14}]$2-DEOXYGLUCOSE UPTAKE AFTER DEAFFERENTATION OF THE MAMMALIAN AUDITORY PATHWAY

- A MODEL FOR EXAMINING TINNITUS

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ABSTRACT

Fifteen adult male guinea pigs were subjected to right cochlea ablation and their central nervous system afferent auditory pathways were examined for metabolic activity at varying time intervals after surgery. The method of examination used was the $[C^{14}]$2-deoxyglucose method of autoradiography of Sokoloff e. al. (41). Within five hours after surgery, metabolic activity in the right cochlear nuclei, left medial accessory superior olive, left lateral lemniscus, left inferior colliculus, and left medial geniculate body, which received afferent input predominantly from the ablated cochlea, was lower than the activity in the corresponding auditory structures on the contralateral, intact, side or in the intact control animal. Metabolic activity returned to the deafferented structures in a rostral to caudal manner; activity returned to the inferior colliculus by 2 days post-op but not to the ventral cochlear nucleus until 14-42 days post-op. The left lateral superior olive showed increased metabolic activity, to a level greater than the intact side, between 9 - 42 days. The dorsal cochlear nucleus had return of metabolic activity at 2,9, and 42 days but no significant return at 5 and 14 days when compared to the acute animal. This complex pattern may be due to the involvement of this nucleus in a binaural afferent-efferent feedback loop. The medial accessory superior olive and medial geniculate did not show any significant return of metabolic activity as late as 42 days post-op.

The hypothesis is that this metabolic activity represents aberrant neuronal activity arising in the auditory pathway

following release of inhibitory influences by deafferentation. This model may provide a pathophysiologic correlate for the study of tinnitus.

INTRODUCTION

Tinnitus, or "ringing in the ears", is a subjective otologic symptom which plagues over 36 million adult Americans and is extremely debilitating to 7.2 million of them (47). According to Fowler (18), tinnitus is present in over 85% of the patients with ear disease and is always accompanied by deafness in the frequency range of the tinnitus. Factors inducing tinnitus are those which lead to a conductive or sensorineural hearing loss and have included loud noise, trauma to the temporal bone, ototoxicity from quinine, sulfonamides, aminoglycosides, and salicylates, otosclerosis, impacted cerumen, imperfect functioning of the eustachian tube, and stapedectomy (18,6,40). The most commonly associated otologic lesion is degeneration of the organ of Corti (6).

Despite its prevalence, attempts at treatment of tinnitus are largely palliative, with many sufferers being told to simply learn to live with their symptoms(46). The prerequisite for a cure is a better knowledge of the pathophysiology of tinnitus, an area which at present is somewhat obscure. The elucidation of the pathophysiology can best be approached by the development of an animal model on which experimental data can be derived. It is the attempt of this work to develop such an animal model.

Some researchers have suggested that tinnitus may be the illusion of sound generated by cortical interpretation of spontaneous neural activity in the auditory pathways (18,6,28,38,13, 32). This neural hyperactivity is hypothesized to result from release of inhibition on the auditory neurons following alteration or interruption of normal afferent activity. The inter-

pretation by the cortex of spontaneous activity in the auditory pathway as sound has an analogue in the visual system, where activity of the neurons may be perceived as light in the absence of actual photostimulation on the retina (31). Spontaneous discharges during some temporal lobe epilepsy have been associated with the subjective perception of sound (6,13) as has direct electrical stimulation of the cochlea (32).

Further support of the theory of aberrant neuronal activity as the source of tinnitus comes from recent success using pharmacological treatments known to suppress neuronal hyperactivity in other parts of the central nervous system, such as lidocaine, carbamazepine, and amylobarbitone (28,27,38,13). Portmann et. al. (32) claim that their success in suppressing tinnitus by electrically stimulating the cochlea may be due to the inhibition of abnormal activity in the sensorineural elements by such peripheral stimulation. Surgery aimed at correcting tinnitus by sectioning the acoustic nerve or cochlea ablation has often failed to provide relief and has even resulted in paradoxical worsening of tinnitus (46,3,17). Such results are in accordance with the above proposed mechanism for tinnitus, however, some researchers claim excellent success rates with eighth nerve section to the extent that the patients deemed their tinnitus "improved" or "cured" (40,33). This improvement is sometimes a change in the character, tone, or perceived loudness of the tinnitus to a more tolerable form without actual alleviation of the phenomenon (33).

The current study entails deafferentation of the auditory pathway of the guinea pig by ablation of the cochlea and subsequent examination of various auditory nuclei. Although it is

difficult to define the presence of subjective tinnitus in an animal, it is hoped that the identification of spontaneous activity in the auditory pathway following deafferentation may provide a pathophysiologic correlate for tinnitus. The method employed for examining the auditory pathway is the [C^{14}]2-deoxyglucose method of autoradiography which observes changes in glucose uptake in actively metabolizing cells (30,41,42,23).

Radioactive [C^{14}]2-deoxyglucose is a sugar which is taken up by cells which normally metabolize glucose. The metabolism of neurons is entirely dependent upon circulating glucose. Once inside the cells, the 2-DG is phosphorylated via the Embden-Meyerhoff pathway (43) but the resultant 2-DG-6 phosphate cannot be further metabolized and is "trapped" within the neuron. The rate of 2-DG accumulation is thus proportional to the rate of metabolic activity of the cell. The 2-DG accumulation in various tissues can be visualized by the method of autoradiography; the tissue section is placed in contact with x-ray film and the radioactive areas are seen as darkened areas of film.

Metabolic activity demonstrated by 2-DG technique is believed to be representative of functional neuronal activity, as a result of numerous studies demonstrating increased 2-DG uptake in the presence of neurophysiologic activity (32,41,42, 23,22,37). For example, electrical stimulation of the sciatic nerve in both rats(23) and cats (22) has been shown to increase 2-DG uptake in the ipsilateral dorsal horn of the spinal cord. In mammalian sensory systems, 2-DG uptake has been seen to increase in focal regions of the olfactory bulb following odor

stimulation (37) and has been used to map ocular dominance and orientation columns in the visual system (20).

During the early trials of the 2-DG method, it was noted that the structures of the auditory system, particularly the cochlear nuclei, superior olivary complex, inferior colliculus, and the medial geniculate bodies, showed high levels of metabolic activity as reflected by high radioisotope uptake compared to other grey matter in the unanesthetized rat exposed to ambient laboratory noise (41,42,23). Occlusion of the external auditory canals lead to bilateral reduction of 2-DG uptake in the auditory structures, while unilateral occlusion resulted in unilateral decrease in the inferior colliculus contralateral to the occlusion (30,23). These findings were felt to demonstrate a high level of metabolic and, hence, neuronal activity in the auditory nuclei which could be reduced by blocking afferent input. The fact that the auditory pathway has such a marked pattern of 2-DG uptake under normal conditions is particularly advantageous for discerning changes after experimental manipulations.

Recent studies have utilized the 2-deoxyglucose method to study the auditory pathway (44,26,35,39,49). Maps of the tonotopic organization of the auditory nuclei have been made which have corresponded well with maps made by conventional electrophysiologic studies (35,39). Studies in which the auditory pathway has been deafferented have demonstrated decreased activity in the corresponding pathway (49,44,26). Tanaguchi and Saito (44) have noted a return of activity with time in the deafferented

inferior colliculus of immature mice, which they attribute to plastic reorganization. The present study will look at the activity in the deafferented auditory pathway of adult mammals and document the pattern of activity in several of the major relay nuclei with time.

The afferent auditory pathway:

The following description of the afferent auditory pathway is based upon recent studies using an array of techniques including neurophysiology, degeneration histology, and autoradiography, in a variety of mammals including cats, rats, and guinea pigs. It should be noted that results obtained in one mammal are not necessarily applicable to the auditory systems of all mammals and the following description, therefore, is merely an approximation based upon the current literature. Figure 18 is a summary illustration of the afferent auditory pathway.

Primary sensory fibers, which contact the hair cells in the organ of Corti, join at the spiral ganglion in the modiolus to give rise to the cochlear nerve (10). The cochlear nerve enters the brainstem at the cerebellopontine angle and each of the fibers bifurcates, sending one terminal branch to the dorsal cochlear nucleus and one to the ventral nucleus (10,9). Both cochlear nuclei are arranged tonotopically according to frequency (10,9).

Secondary fibers arising in the cochlear nuclei enter one of three striae which cross the midline and ascend in the contralateral lateral lemniscus without synapse (10). Some of the

fibers arising in the ventral cochlear nucleus and travelling in the ventral acoustic stria, give off terminations to the ipsilateral and contralateral superior olives, accessory medial superior olives, and trapezoid bodies, providing binaural input to these nuclei (10,11,9,16). Tertiary fibers from these nuclei predominantly ascend in the contralateral lateral lemniscus (10) although recent studies suggest that tertiary fibers from the lateral superior olives are equally distributed in both ipsilateral and contralateral lateral lemnisci in cats and rats (15,1,4). The tertiary fibers from the medial accessory superior olives are believed to ascend only in the ipsilateral lateral lemniscus (15,4).

Most fibers of the lateral lemniscus ascend to the midbrain where they terminate in the inferior colliculus. The inferior colliculus is tonotopically organized with neurons responding to a low best frequency in the dorsolateral segment and neurons with a high best frequency in the ventromedial aspect (34,4,1). Some fibers cross the midline in the commisure of the inferior colliculus to synapse with the contralateral structure (10).

Fibers originating in the inferior colliculus ascend via the brachium of the inferior colliculus to the thalamus where they terminate in the ipsilateral medial geniculate body. A small percentage of projections terminate in the contralateral medial geniculate body(2). Fibers from the medial geniculate project to the temporal cortex.

An efferent auditory pathway has been shown which forms a feedback loop from the bilateral superior olivary complexes to

the cochlea, via the dorsal cochlear nucleus, to modify sensory input (10,14,9). This pathway is believed to participate in binaural comparison of input at the level of the lateral superior olives to facilitate sound localization.

METHODS

Nineteen adult male guinea pigs weighing 250-690 g were divided into six experimental groups and four control groups. The experimental groups received unilateral cochlea ablation under aseptic conditions and ketamine anesthesia with local subcutaneous and intramuscular injection of 1% lidocaine with epinephrine. Using 20X magnification, the mastoid bulla of the temporal bone was approached via an incision posterior to the pinna. Fracturing the lateral wall of the mastoid bulla revealed the fused incus-malleus, which was removed. Visualization of the cochlea was achieved through an opening in the wall of the middle ear and the cochlear nerve was macerated by out-fracturing of the lateral cochlear wall (8). The cavity was filled with Gelfoam (Upjohn)and the wound closed in two layers. Neosporin ointment (Burroughs Wellcome) was applied sparingly along the external suture line and the guinea pig was placed in a soundproofed box (Industrial Acoustics Co.,N.Y.) for recovery.

After surgery, the animals recovered for varying intervals of time before being sacrificed and examined using the [C^{14}]2-deoxyglucose method of autoradiography. Two of the guinea pigs were sacrificed 2 1/2 - 4 1/2 hours following cochlea ablation, three animals at two days after surgery, two animals at five days, three animals at nine days, two animals at fourteen days, and three animals at forty-two days. During the period of time between surgery and sacrifice, the guinea pigs lived in individual cages within the soundproofed boxes in a quiet laboratory. However, the soundproofed boxes were latched 1-2 cm ajar, at times there were three cages within

a single box, and the animals were therefore exposed to noise from one another as well as the sounds of doors and cabinets being closed, water running, and footsteps in the laboratory.

At the time of sacrifice, the guinea pigs were injected intraperitoneally with 10 uCi / 100 g body weight of [C^{14}] 2-deoxyglucose (New England Nuclear, 52.2 mCi/mM) and then placed in a soundproofed chamber (IAC) with white noise (5 Hz - 500 kHz, Gaussian Noise Generator, Elgenco Inc., Calif.) at a sound level of 70 - 73 dBC , monitored continously with a sound level meter. After 90 minutes, they received an overdose of pentobarbital and their brains were rapidly removed and frozen in isopentane chilled with dry ice. The brains were then sectioned in a cryostat at -10 °C and the sections were rapidly thawed onto coverslips and placed in contact with x-ray film (Kodak SB-5) for autoradiography. The sections were later placed in contact with mammography film (Kodak MR-1) for autoradiographs with finer resolution.

The control group consisted of four animals: (1) one intact control which was exposed to [C^{14}] 2-deoxyglucose under the same conditions as the experimental animals but with no surgery, (2) an anesthetized guinea pig which was injected with 2-deoxyglucose while under the influence of ketamine at a level of anesthesia equivalent to that achieved during surgery, but no surgery was performed, and (3),(4) two "sham-operated" animals. The sham operations were performed in the same manner as the experimental surgery and involved fracturing through the mastoid bulla to expose the middle ear, packing the middle ear

with Gelfoam, and closing the wound. In the sham operations, unlike the experimental procedures, the ossicles were not removed and the inner ear and cochlea were left intact. One sham-operated animal was sacrificed 17 hours after surgery, the other was sacrificed 42 days post-operatively.

Data analysis

5 X 7" photographs were made from the autoradiographs of brain sections and examined visually to determine relative uptake of [C^{14}]2-deoxyglucose by various structures along the bilateral afferent auditory pathways. As a control for inter-animal differences in metabolism and uptake of 2-DG, the deafferented auditory pathway was compared to the contralateral intact pathway within a given animal.

The photographs were further analyzed with the aid of a reflectance densitometer (KD320 Fotonic Sensor, Mechanical Technology Inc., N.Y.) and computer which generated a tracing corresponding to the relative intensities of radioisotope accumulation on a scan line across a given brain section. A peak on the tracing corresponds to an increased uptake of 2-DG by the tissue. Each auditory structure was thereby represented as a peak in the intensity plot. Quantitative assessment of the 2-DG uptake was accomplished by calculation of the area under the peaks of the intensity tracing; this rough approximation of 2-DG accumulation should not be confused with the calculations of Sokoloff et. al. involving direct measurements of plasma glucose and 2-deoxyglucose concentrations, blood flow rates, etc. (41, 42).

Three different scan lines were made across the auditory structures in a given brain section in order to minimize sampling bias from a single scan line. The areas under the peaks of the resultant intensity tracings were then calculated by computer for each of the three scans. Thus, for each auditory structure in a given brain section, three area calculations were derived, providing an approximation of the 2-DG uptake in that structure.

In order to control for inter-animal differences in metabolism and absorption of 2-DG as well as differences in darkness based on photographic technique, the deafferented auditory pathway was compared to the contralateral intact pathway within a given scan. This comparison assumed the form of a ratio of the area under the peak corresponding to the intact auditory nucleus to the area under the peak corresponding to the contralateral deafferented auditory nucleus in the same scan. Since three scans were obtained for each set of auditory structures, three area ratios were derived for a given auditory nucleus in a particular animal. These three ratios for each animal were summated with the ratios derived for the other animals in an experimental group to yield an average ratio and standard deviation for a particular auditory structure in a set of animals.

The average ratios for an auditory nucleus could be compared across all of the six experimental groups by means of an F-test for the one way analysis of variance. If the F-test indicated that the mean ratios were significantly different across the six experimental groups, a Student's T-test was employed to compare the average ratio of one group to another.

Histology :

Following right cochlea ablation and removal of the brain for sectioning, the guinea pig skulls were preserved in 10% phosphate buffered formalin. After three weeks in formalin, they were decalcified in 5% formic acid, embedded in paraffin, and sectioned at 10 microns. Every tenth section was stained with hematoxylin and eosin and examined for comparison of the operated inner ear to the intact side.

RESULTS

Control:

The pattern of (C^{14}) 2-deoxyglucose uptake in the auditory pathway of a nonoperated control guinea pig is illustrated in Figure 1 A -D, which consists of photographs made from autoradiographs of selected brain sections. In all of the illustrations,the right side of the photograph corresponds to the right side of the brain. Figure 1A is a section through the medulla; the arrows identify the ventral and dorsal cochlear nuclei. Note that there is no appreciable difference in density , and hence 2-DG uptake, between the set of nuclei on the right and that on the left. Figure 1B shows a section through the level of the pons with the superior olivary nuclei and medial accessory superior olivary nuclei indicated. At this level, the two sides also show similiar densities with regard to 2-DG uptake. Figure 1C of the midbrain again demonstrates no difference between right and left sides in the inferior colliculi, as does Figure 1D at the level of the medial geniculate bodies of the thalamus.

Figure 2 is a set of computer-generated graphs plotting density versus distance along a scan line. These plots were generated by moving the probe of a reflectance densitometer along the line crossing the auditory structures as indicated in the photographs seen on the right of Figure 2. Note that the peaks in the computer plot correspond to the regions showing increased densities of 2-DG in the brain section, with particularly prominent uptake appearing in the cochlear nuclei (Figure 2A). Also observe that the tracing is symmetrical with respect to the cochlear nuclei on the left and those on the right. Confirmation of this observation is

provided by comparison of the areas under the peaks. A ratio of the areas under the peaks on the left side to the areas under the peaks on the right side, across three tracings, yields a mean ratio of 1.23 ± 0.57 standard deviation for the dorsal cochlear nuclei and 0.65 ± 0.14 S.D. for the ventral cochlear nuclei. These ratios approximate the expected ratio of 1:1 based on visual inspections of the brain section in this normal control.

Figure 2B is a plot of the intensities of 2-DG uptake across the superior olives. The ratios of the areas under the peaks of the right to left sides are 1.25 ± 0.33 S.D. for the superior olives and 0.79 ± 0.14 S.D. for the medial accessory olives. These figures also approximate one. Figure 2C shows the intensities across the inferior colliculi. The ratio of the area under the right peak to the area under the left peak is 1.09 ± 0.30 S.D., reaffirming the symmetrical appearance on visual inspection. The density of 2-DG in the medial geniculate bodies is represented in Figure 2D. Here, too, a ratio of the area under the right curve to that under the left is close to unity : 0.95 ± 0.19 S.D.

Anesthetized unoperated control :

The results obtained for the anesthetized unoperated animal were not significantly different from those for the intact control when mean ratios of area under the intensity peaks were compared for each of the auditory structures (Student's T-test, $p>0.10$).

Animals sacrificed 2-5 hours after right cochlea ablation (Acute):

Typical results from animals sacrificed 2 1/2 - 4 1/2 hours after right cochlea ablation are depicted in Figures 3 and 4. Figure 3A is a section showing the cochlear nuclei; the right side correspon to the deafferented auditory pathway at this level. It is apparent

that the density of the right cochlear nuclei (arrow) is lower than that on the intact left side. Figure 4A, the computer plot of intensity across the same section , reinforces this observation of a marked discrepancy in 2-DG uptake between the operated and intact sides. The ratio of areas under the peaks of the left cochlear nuclei to the areas under the peaks of the right cochlear nuclei is 10.11 ± 3.66 S.D. for the dorsal cochlear nucleus and 9.99 ± 5.30 S.D. for the ventral cochlear nucleus, both values being substantially greater than the 1:1 ratio for the control animal. This reduction in 2-DG uptake on the deafferented side when compared to the control is significantly different at the 0.01 level by Student's T-test.

Figure 3B depicts the superior olivary complexes of the same animal. It is evident by examination that the 2-DG uptake of the left medial accessory olive (arrow), which corresponds to the site of the predominant connections from the deafferented side , is less than the uptake of the right medial olive. The right and left lateral superior olivary nuclei appear to be of equivalent densities. Comparison of the areas under the corresponding intensity peaks in Figure 4B provides quantitative evidence that the left medial accessory olive has significantly less 2-DG uptake than the control at the 0.05 level and that there is no significant difference between the 2-DG uptake in the lateral superior olives of this experimental animal and that of the control. The ratio of the areas under the peaks of the left olivary nuclei to the areas under the peaks of the right olivary nuclei is 12.87 ± 7.50 for the medial accessory superior olives and 1.13 ± 0.49 for the lateral superior olives.

This trend of diminished 2-DG accumulation in the deafferented auditory nuclei continues in the more rostral structures, the inferior colliculi and medial geniculate bodies (Figures 3C & D). At the level of the inferior colliculi , the ratio of the area under the intensity curves (Figure 4C) on the left, intact, side to that on the right side is 6.68 ± 2.09, a value significantly greater than one and, hence, different than control ($p<0.01$). For the medial geniculate bodies (Figure 4D), the mean ratio is 1.51 ± 0.74, which is significantly different than control at the 0.05 level. It should be noted that the density of 2-DG uptake in the medial geniculate bodies (Figure 4D) is low compared to background uptake, making comparisons between sides difficult.

Animals sacrificed 42 days following right cochlea ablation :

The pattern of diminished 2-DG accumulation in the deafferented auditory pathway in animals examined within several hours of cochlear nerve section can be contrasted to the 2-DG uptake in guinea pigs examined 42 days following cochlea ablation. Figure 5 A-D is a collection of photographs of brain section autoradiographs from such a 42 day animal and Figure 6 A-D is a set of the density tracings. The cochlear nerve section was performed on the right and again the deafferented ascending auditory pathway is delineated by arrows; the right cochlear nuclei (Fig. 5A), the left superior and accessory olives (Fig. 5B), the left inferior colliculus (Fig. 5C), and the left medial geniculate (Fig. 5D).

At the level of the cochlear nuclei (Fig.5A), both the right dorsal and ventral nuclei appear to be considerably darker than the right nuclei in the animal sacrificed several hours after deafferentation (Fig. 3A). Comparison of the intensity tracings for the 42 day animal (Fig.6A) and 1 day animal (Fig.4A)

support this observation; simple inspection reveals higher peaks for the right nuclei, particularly the ventral nucleus, in the 42 day animal than in the 1 day animal. After correction for inter-animal differences in basal metabolic rates and 2-DG uptake by comparing the area under the peaks on the intact side to the area under the peaks on the lesioned side within an individual animal, it is still apparent that the 42 day animal has significantly more 2-DG uptake on the deafferented side than the one day animal ($p<0.01$ for both dorsal and ventral cochlear nuclei). The average ratio for the 42 day animal is 2.37 ± 0.74 for the dorsal cochlear nucleus and 1.43 ± 0.82 S.D. for the ventral cochlear nucleus, values considerably closer to 1:1 than the ratios for the acute animal. In fact, the 2-DG level in the right ventral cochlear nucleus has returned to control levels (no significant difference at 0.10 level by T-test) although the 2-DG uptake in the right dorsal cochlear nucleus is still significantly less than control ($p<0.025$).

Figure 5B shows the superior olivary complexes in the 42 day animal. Even at 42 days following deafferentation, the left medial accessory olive appears to have little 2-DG uptake when compared to the right side. This lack of uptake is not significantly different from the situation in the one day animal ($p>0.10$); the average ratio is 8.16± 5.04. The left lateral superior olive, however, appears slightly darker than its contralateral counterpart. This observation is confirmed by the higher intensity peak generated by the left superior olive than the right in the

density scan shown in Figure 6B. The ratio of the area under the right lateral peak to the area under the left lateral peak is 0.71 ± 0.17, a value less than the 1:1 ratio seen for both the control and one day post-op animals ($p < 0.05$).

The inferior colliculi of the 42 day animal (Figure 5C) demonstrate the most striking contrast when compared to the one day animal (Figure 3C). Note the marked increase in 2-DG uptake on the left, deafferented side when compared to the one day animal. The ratio of the area under the right peak to the area under the left peak (Figure 6C) is 2.93 ± 0.89 ,a value significantly different than the acute ratio of 6.68 ± 2.09 ($p < 0.01$). Thus, the 2-DG uptake in the deafferented inferior colliculus is significantly greater than that in the acute animal but not as great as that in the control.

The intact side still has greater 2-DG uptake than the deafferented side at the level of the medial geniculate bodies (Fig. 5D) ($p < 0.025$ when compared to control). The deafferented side does not appear to have any greater uptake after 42 days than after one day (no significant difference at 0.10 level). Again, however, it should be noted that the 2-DG uptake in both medial geniculate bodies is not appreciably higher than the background and therefore careful comparisons are difficult to make (Fig.6D).

Figure 7 is a graph which summarizes the findings when the 42 day animal is compared to the acute, 1 day animal. In this figure, the ordinate represents the ratio of areas under the intensity peaks of the intact side to the operated side, one being the value of the control. Thus, a ratio greater than one signifies decreased 2-DG uptake on the deafferented side, a value of one means equivalent uptake on both sides, and a value

less than one denotes increased uptake on the deafferented side. It can be noted that for all of the auditory structures except the medial accessory superior olives and the medial geniculate bodies, the 42 day animal had greater 2-DG uptake on the deafferented side than the 1 day animal. At the level of the lateral superior olives, this increased uptake was even greater than the uptake seen in the control animal. In the medial accessory superior olives and medial geniculate bodies, the uptake on the lesioned pathway was equally diminished for one day post-op and 42 days post-op.

Animal sacrificed two days following right cochlea ablation:

The pattern of 2-DG uptake two days after destruction of the auditory receptor organ is displayed in Figures 8 and 9. Two days post-op there is the same degree of reduction of 2-DG uptake in the deafferented ventral cochlear nuclei, superior olives, and medial geniculate bodies as several hours post-op ($p > 0.10$). However, in the dorsal cochlear nucleus and the inferior colliculus there is significantly greater 2-DG uptake in the lesioned side at 2 days than there was at 2-5 hours ($p < 0.025$). Inspection of Figure 8C reveals a sharp band of increased density in the dorsolateral , low frequency portion of the left inferior colliculus surrounded by a slightly increased uptake in the rest of the structure. This dorsolateral band of increased uptake was seen in two out of three two day animals and is demonstrated in the intensity curve of Figure 9C as a sharp peak. Measurement of the areas under the curves indicates that the uptake on the left is still not equivalent to the uptake on the right side.

Animal sacrificed five days after right cochlea ablation :

A typical collection of results from an animal examined 5 days after right cochlea ablation is presented in Figures 10 and 11. At all levels except the inferior colliculus, this five day animal shows the same decrease in 2-DG uptake on the lesioned side as the acute, one day animal ($p > 0.10$). The deafferented inferior colliculus (Figure 10C) has a broad band of increased density surrounded by moderately increased background density in the dorsolateral portion of the structure. This 2-DG uptake is significantly greater than that seen in the acutely lesioned animal ($p < 0.10$) but is not equal to the level of 2-DG uptake in the intact side (Figure 11C). The dorsolateral band of increased activity was consistent for both five day animals.

Animal sacrificed nine days after right cochlea ablation :

Figures 12 and 13 represent the results from an animal sacrificed 9 days following right cochlear ablation. Reduction in radioisotope uptake on the lesioned side was not significantly different from that seen for the acute animal in the ventral cochlear nucleus, the medial accessory olives, and the medial geniculate bodies (p 0.10). The 2-DG uptake on the lesioned side was significantly increased in the nine day animal as compared to the acute level of the dorsal cochlear nuclei ($p < 0.025$) (Figure 12A), lateral superior olives ($p < 0.025$) (Figure 12B), and inferior colliculus ($p < 0.01$) (Figure 12C). The left lateral superior olive was actually darker than the intact side ($p < 0.025$) (Figure 13B). The 2-DG uptake in the deafferented inferior colliculus consisted of a dense dorsolateral band of increased metabolic

activity, surrounded by a moderately increased density in the rest of the structure (Figure 12C), although the total amount of uptake on the lesioned side was not equivalent to that on the intact side (Figure 13C). The dorsolateral band of increased activity was noted in all three of the nine day animals.

Animal sacrificed 14 days after right cochlea ablation :

Typical results for a guinea pig 14 days after right cochlea ablation are presented in Figures 14 and 15. The 2-DG uptake is reduced on the lesioned side at the cochlear nuclei and superior olives to the same extent as in the 2-5 hour survival animal ($p<0.05$). It should be noted , however, that there is a dark spot of increased metabolic activity at the right ventral cochlear nucleus (Figures 14 A&B) which is darker than in the acute animal at a level of borderline significance ($0.10>p>0.05$). The inferior colliculus of the 14 day animal shows significantly greater 2-DG uptake than the acutely sacrificed animal ($p<0.05$), however, in this animal the band of increased activity is in the ventromedial, high frequency , portion of the colliculus (Figure 14C) as opposed to the dorsolateral band in the 2,5, and 9 day animals. This ventromedial band was not consistent for both 14 day animals; the other 14 day aniaml exhibited a dorsolateral band of increased activity in the inferior colliculus. The deafferented medial geniculate body of the 14 day animal has significantly less 2-DG uptake than the acute animal ($p<0.05$).

A summary graph of the time course of changes in 2-DG uptake with deafferentation is presented in Figure 16. The ordinate represents the ratio of the area under the intensity peak for the intact structures to the area under the peak

for the deafferented structures. A ratio of one to one means equal 2-DG uptake bilaterally, a ratio greater than one means greater 2-DG uptake on the intact side than the deafferented side, and a ratio of less than one represents greater 2-DG uptake on the deafferented side than the intact side. The abscissa represents time in days after right cochlea ablation. Values that are not significantly different than each other share the same pattern of cross-hatching.

Figure 16 demonstrates the following :

(1) In the ventral cochlear nuclei, the metabolic activity on the lesioned side is significantly reduced and remains so until some time between 14 and 42 days post-op, when metabolic activity returns to a level comparable to that in the unlesioned animal.

(2) In the dorsal cochlear nucleus, metabolic activity is reduced acutely following deafferentation but its pattern of return is variable. In animals at 2,9, and 42 days, there is a significant return of metabolic activity, although not to the level in the intact side. However, at 5 and 14 days , there is no significant return of activity when compared to the actue animal.

(3) In the medial accessory superior olives, metabolic activity is sharply reduced with cochlear ablation and does not return to any significant degree within 42 days.

(4)In the lateral superior olives, the metabolic activity remains unchanged on either side following cochlea ablation until some time between 9 - 42 days. During this interval, there is actually an increase in metabolic activity on the deafferented side as compared to the intact side.

(5) At the level of the inferior colliculus, metabolic activity drops markedly following deafferentation but begins to return between 1 - 2 days. This return takes the form of dorsolateral bands of increased metabolic activity from 2 - 9 days, either a ventromedial band or a dorsolateral band at 14 days, and a fairly uniform increase across the structure at 42 days.

(6) The metabolic activity of the medial geniculate bodies is barely above the level of background thalamic activity and therefore it is difficult to compare the two sides quantitatively. However, it is apparent that there is a sharp drop in metabolic activity on the deafferented medial geniculate body in 2 - 5 hours after surgery which is maintained at least until 42 days.

Sham operated animals :

The "sham operated" animal sacrificed less than one day after surgery had significantly diminished 2-DG uptake on the "sham operated" side at all levels except the ventral cochlear nucleus and lateral superior olives when compared with control ($p<0.05$) (Figure 17). The 2-DG uptake in the "sham operated" inferior colliculus, however, was significantly greater than the uptake in the deafferented inferior colliculus in the guinea pigs examined less than one day after cochlea ablation ($p<0.01$). Examination of an animal 42 days after " sham operation" (Fig. 17) revealed a return of metabolic activity in the sham operated pathway at the level of the dorsal cochlear nuclei ($p<0.01$) and inferior colliculus ($p<0.025$) when compared to the acute "sham operated" animal. There was no significant return of

metabolic activity at the level of the medial superior olives and the medial geniculate bodies.

Histology :

Preliminary histological studies of two decalcified guinea pig skulls following right cochlea ablation revealed that the ablation procedure in these two animals destroyed all but a portion of the basal turn of the cochlea.

DISCUSSION

In the present study, the [C^{14}]2-deoxyglucose method of autoradiography has been used to examine the changes in neuronal activity at various times after cochlear ablation in the guinea pig. This study has confirmed and extended in the adult guinea pig observations made by Tanaguchi and Saito (44) that activity in the deafferented inferior colliculus returns with time in the immature mouse, and has extended the observations to other structures in the auditory pathway. Return of activity occurs as early as two days after deafferentation and in general appears to assume a rostral to caudal pattern; activity returns in the inferior colliculus at two days and in the ventral cochlear nucleus at about 42 days. The medial geniculate bodies and medial accessory superior olives never show a return of activity, while the dorsal cochlear nucleus and lateral superior olives demonstrate complex patterns of uptake that may reflect their involvement in binaural afferent - efferent feedback loops.

The return of metabolic activity observed here may be a representation of functional neuronal activity or it may reflect other changes following deafferentation. A recent study looking at motor nuclei demonstrates that 2-DG uptake is increased as early as 24 hours following peripheral transection of the facial and hypoglossal nerves and attributes this increased metabolism to axonal reaction and regeneration (25). Studies examining ultrastructural changes in the cat medial trapezoid nucleus at various times following cochlear destruction (29,21) noted transneuronal degeneration at the level of this secondary relay nucleus. Changes occurring as

early as 2 - 4 days after deafferentation included hyperplasia of neurofilaments and, by seven days, proliferation of glial phagocytic elements was noted. Degenerative changes had decreased by 56 days and were absent by seven months (21). Another study, looking at guinea pigs with the light microscope and degeneration stains, noted signs of degeneration appearing at eight days in the cochlear nerve bundle but no appearance until two weeks in the ventral cochlear nucleus and three weeks in the dorsal cochlear nucleus, following organ of Corti destruction (48).

These studies suggest the possibility that the increased 2-DG uptake seen in the present study may in fact be due to increased metabolism in regenerating nerves and proliferating glial elements. However, several points speak against this. Although the study by Morest and Jean-Baptiste (29) raises the possibility of trans-neuronal degeneration, such degenerative changes would presumably ascend from the peripheral point of damage. In the present study, increased metabolic activity is noted to appear in a descending, rostral to caudal, direction with the inferior colliculus showing activity as early as two days. The ventral cochlear nucleus does not demonstrate increased activity until sometime between two weeks and six weeks. Since this nucleus gives origin to the fibers of the medial trapezoid body, increased metabolism associated with degenerative changes would be expected to appear at an earlier time in accordance with the Morest and Jean-Baptiste study (29). This time course does, however, correspond to the occurrence of degeneration in the ventral cochlear nucleus in the study by Webster and Webster (48).

We have begun a study to elucidate the histology with

respect to degenerative changes in an animal sacrificed six weeks after deafferentation, at a time when increased 2-DG uptake is noted in the cochlear nuclei, lateral superior olive, and inferior colliculus. Future studies will also look at the 2-DG pattern in an animal allowed to survive a year after deafferentation; by that time the proliferative changes associated with degeneration should be absent (48) and increased 2-DG uptake would presumably be a reflection of functional neuronal activity.

Another possinility suggested by the appearance of bands of 2-DG uptake in the inferior colliculus as early as two days after cochlear ablation is that the organ of Corti was incompletely destroyed and the observed activity relates to the surviving afferent fibers. The preliminary histology confirms that a portion of the basal turn of the cochlea may have been spared by the current method of ablation and we have not yet characterized the integrity of the organ of Corti in the remaining basal segment. Nevertheless, the dorsolateral band of activity seen within 2 to 14 days after ablation corresponds to the area of neurons responding to a low best frequency (34,4,1,36) whereas fibers arising from the basal turn of the cochlea respond to a high best frequency (10). The present surgical approach through the mastoid bulla is constrained by the anatomy such that incomplete destruction of the basal turn remains a possibility (8).An alternative approach would be to utilize an aminoglycoside to destroy the cochlea since with these drugs destruction proceeds from the basal end toward the apex (12).

Since many of the auditory nuclei receive bilateral afferents to some degree, the possibility exists that activity seen in

the deafferented nuclei is actually normal input from the contralateral intact structures which is usually masked. Tanaguchi and Saito(44) have found that transection of the commissure of the inferior colliculus, a fiber tract connecting the two nuclei, did not appreciably alter the return of activity on the deafferented side. Preliminary studies in our laboratory entailing bilateral cochlea ablation yielded the same degree of return of activity on both sides as that seen in the unilaterally deafferented pathway.

It is our contention that the increased metabolic activity seen following deafferentation represents aberrant neuronal activity which arises when the auditory pathway is released from inhibitory influences. It is hypothesized that these inhibitory influences require intact afferent activity for their integrity. This spontaneous neuronal activity in the auditory pathway following cochlear ablation may provide a pathophysiologic model for tinnitus. Future studies are needed to confirm the presence of spontaneous neuronal activity by electrophysiology and to see if spontaneous activity can be generated by other tinnitus provoking conditions such as ototoxic pharmacologic agents, acoustic trauma, and conductive hearing loss. Finally, this model provides a system upon which potential tinnitus controlling agents such as carbamazepine and other neuronal suppressants can be tested and preventative or curative agents developed.

The finding that "sham-operated" animals showed diminished uptake on the operated side acutely with some return of activity by 42 days post-op is in agreement with the proposed hypothesis because the "sham-operation" probably entailed

some degree of conductive hearing loss. Since removal of the ossicles creates a 40 -50 dB hearing loss (8), packing Gelfoam around the ossicles , as occurred in the sham operation, probably entails a similiar degree of conductive loss by inhibiting their functional monility. This mechanism of functional deafferentation is similiar to that seen with otosclerosis, a fibrotic disease of the middle ear which inhibits ossicle mobility and often leads to tinnitus (18). Future studies are needed to compare larger numbers of animals deafferented via conductive hearing loss to those deafferented by sensory receptor loss to ascertain whether differences exist in the actual pattern of spontaneous activity, our proposed correlate for tinnitus.

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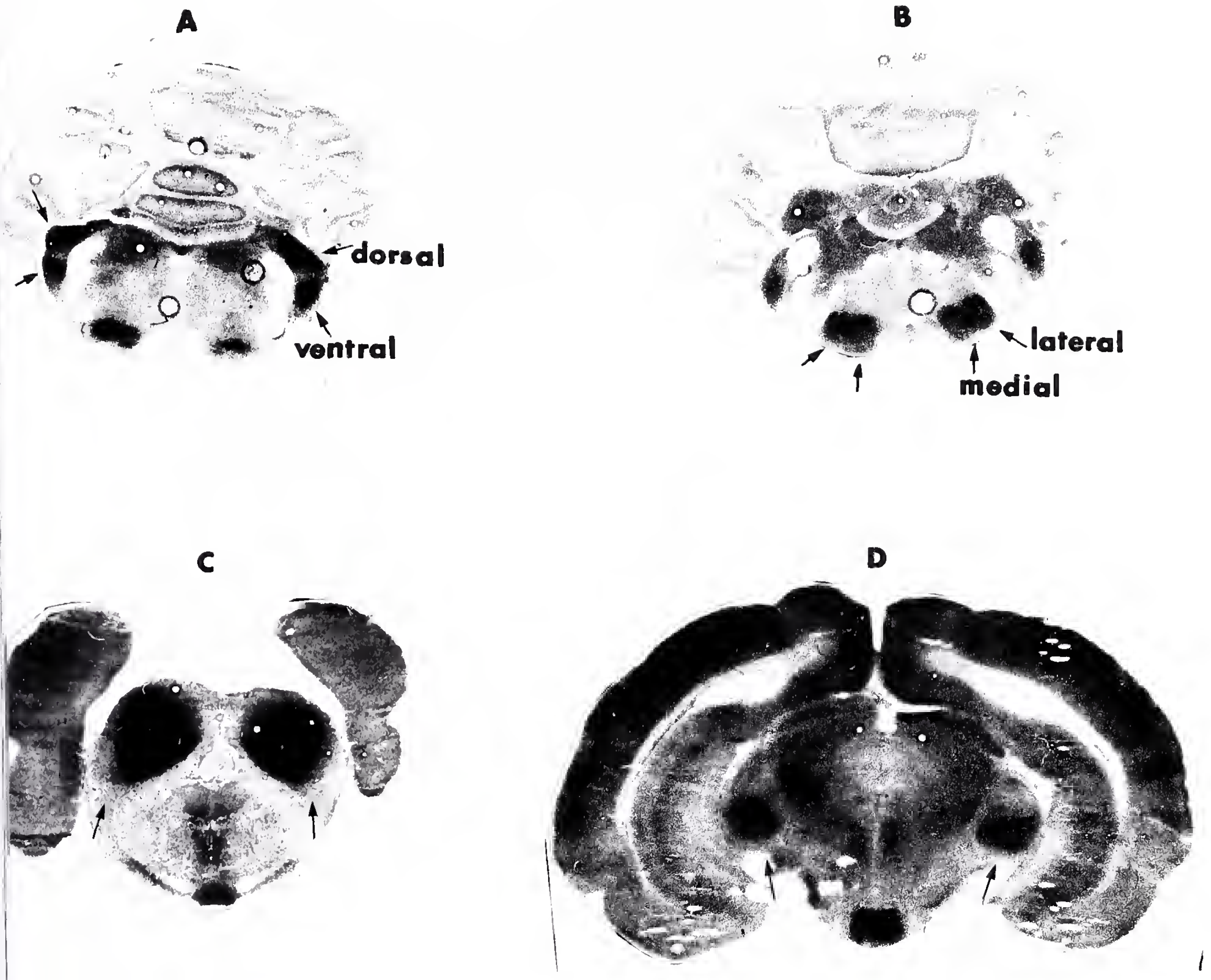


GURE 1. A: 2-Deoxyglucose autoradiograph of guinea pig brain the level of the cochlear nuclei observed in an intact control imal. B: section at the level of the superior olivary nuclei. section at the level of the inferior colliculi. D: section the level of the medial geniculate bodies.

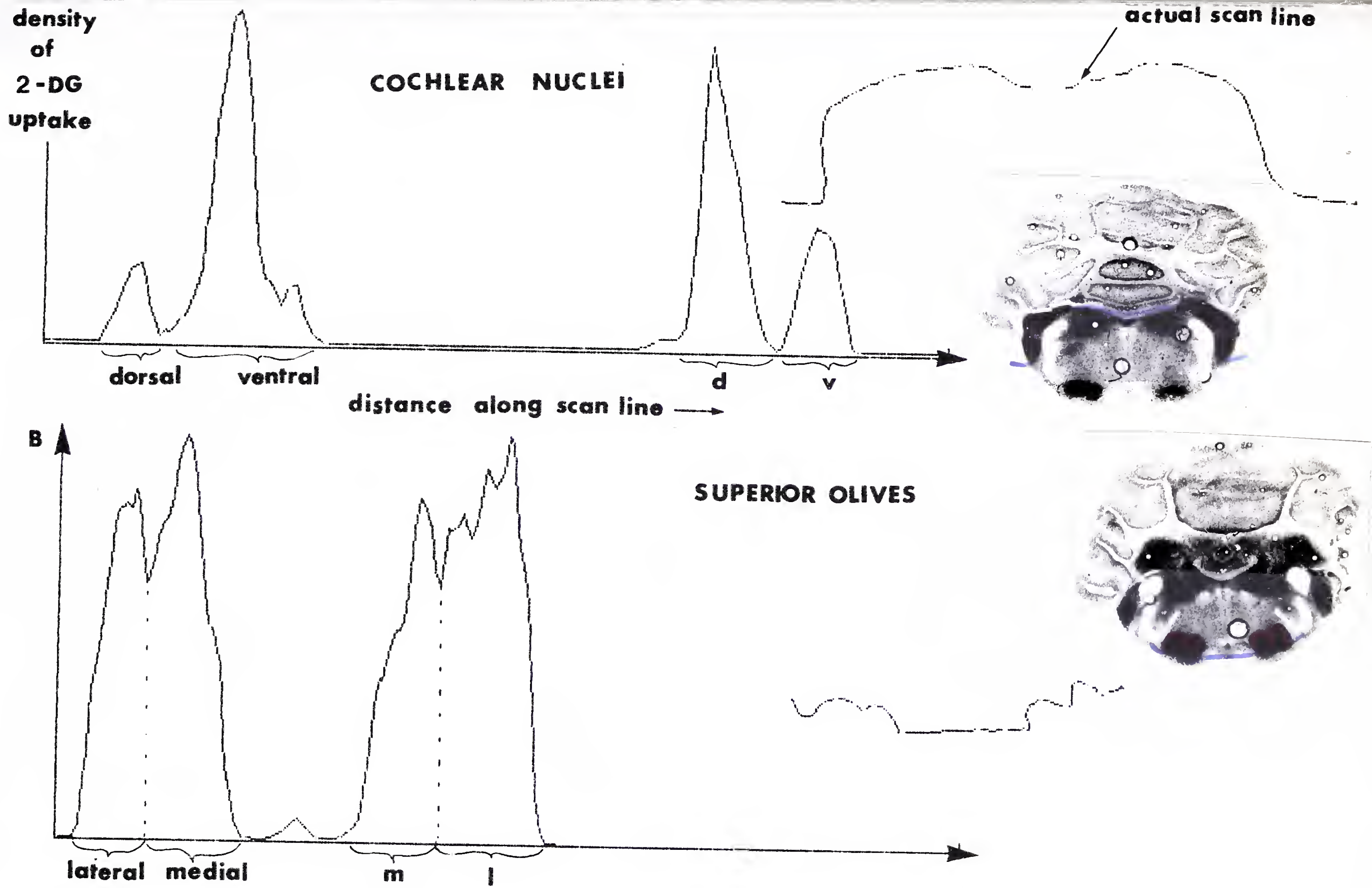


FIGURE 2. Computer -generated graphs plotting density of 2-DG uptake versus distance along a scan line for autoradiographs of brain sections from an intact control animal. These plots were generated by moving the probe of a reflectance densitometer along the line crossing the suditory structures, as indicated in the photographs to the right of the graphs. A: level of the cochlear nuclei. B: level of the superior olivary nuclei.

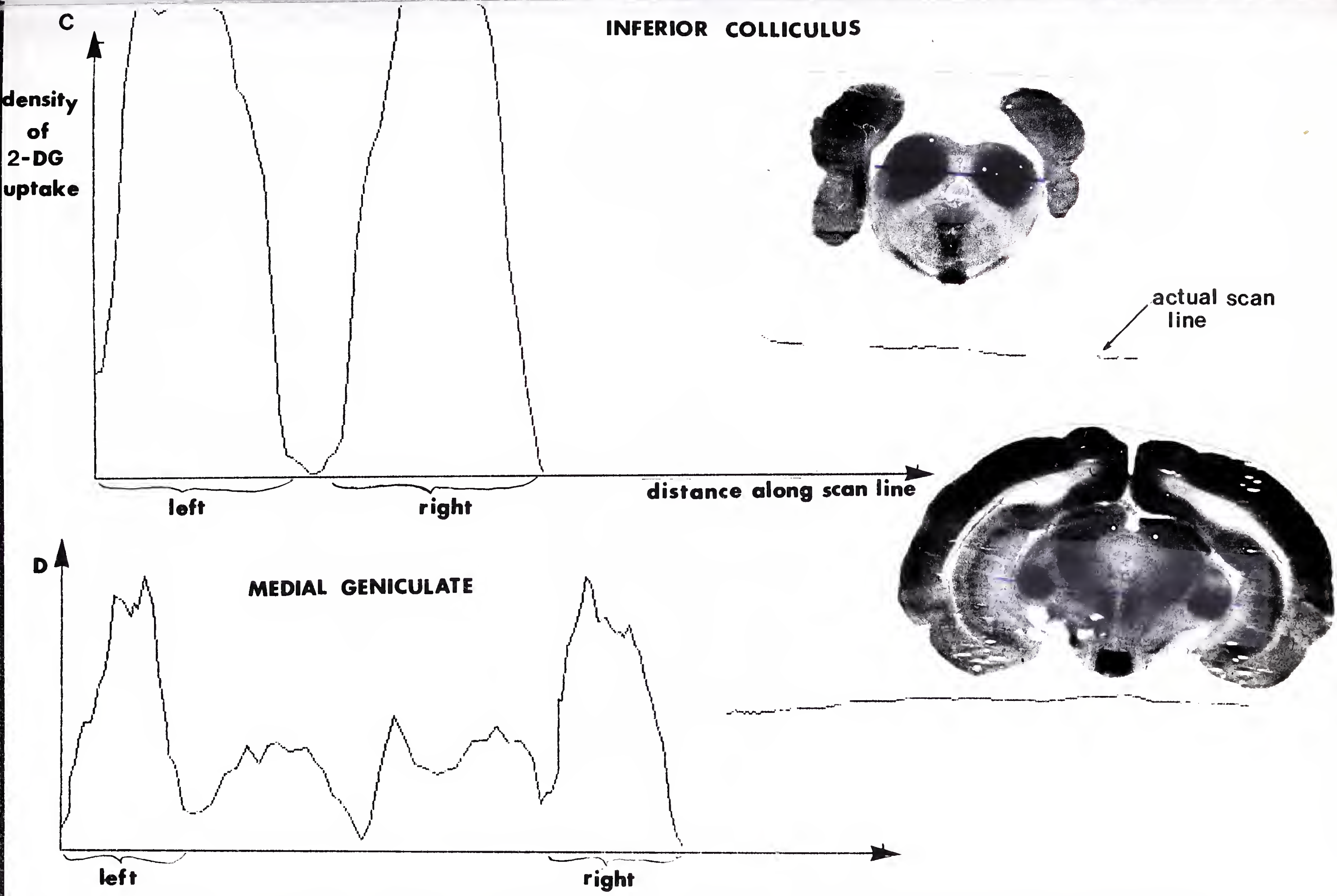


FIGURE 2 continued. C:level of the inferior colliculi. D:level of the medial geniculate

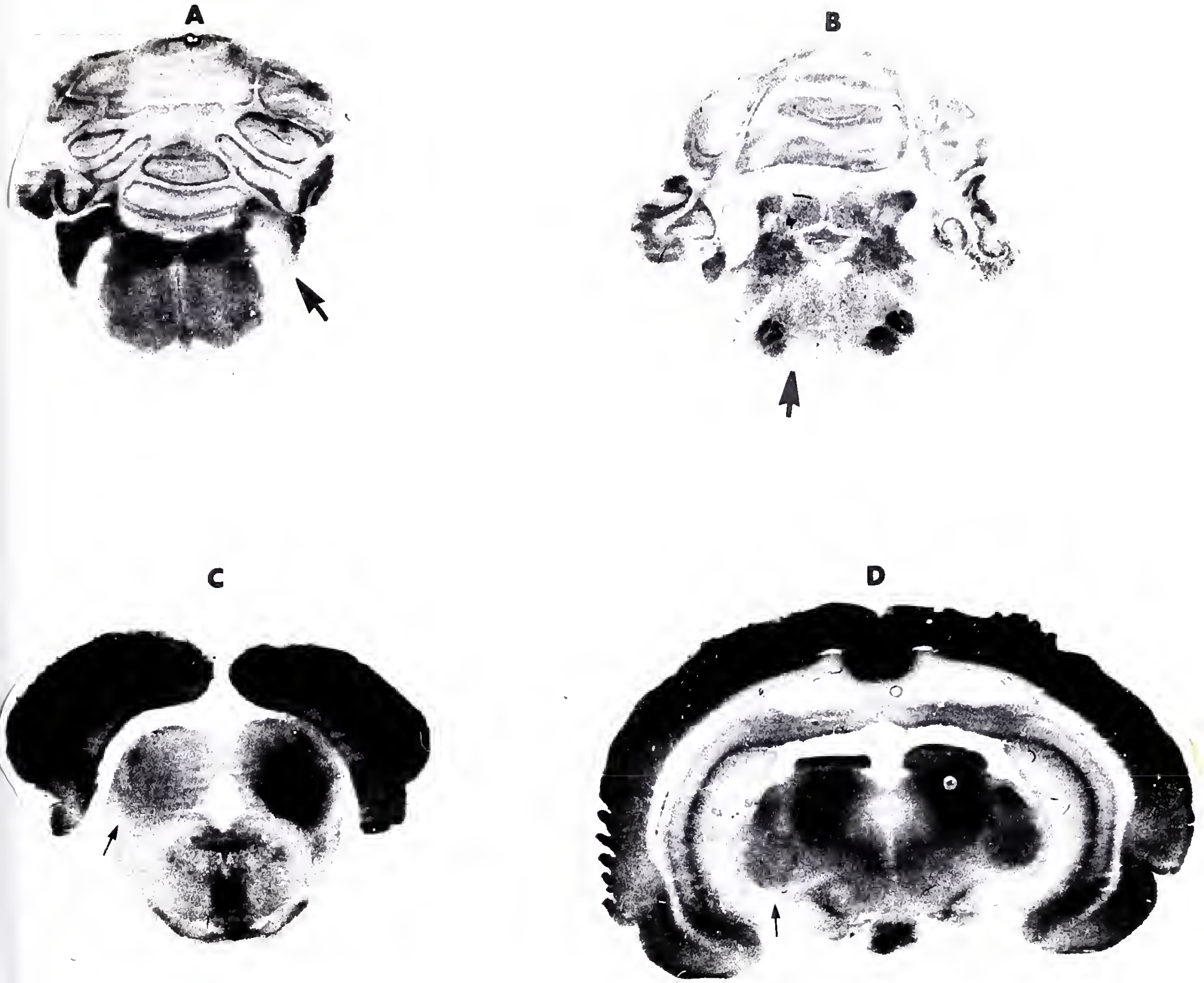


FIGURE 3. A: 2-Deoxyglucose autoradiograph of guinea pig brain at the level of the cochlear nuclei observed 2 - 3 hours after right cochlea removal. B: section at the level of the superior olivary nuclei. C: section at the level of the inferior colliculi. D: section at the level of the medial geniculate bodies. In each photograph arrows indicate sites of reduced uptake compared to control.

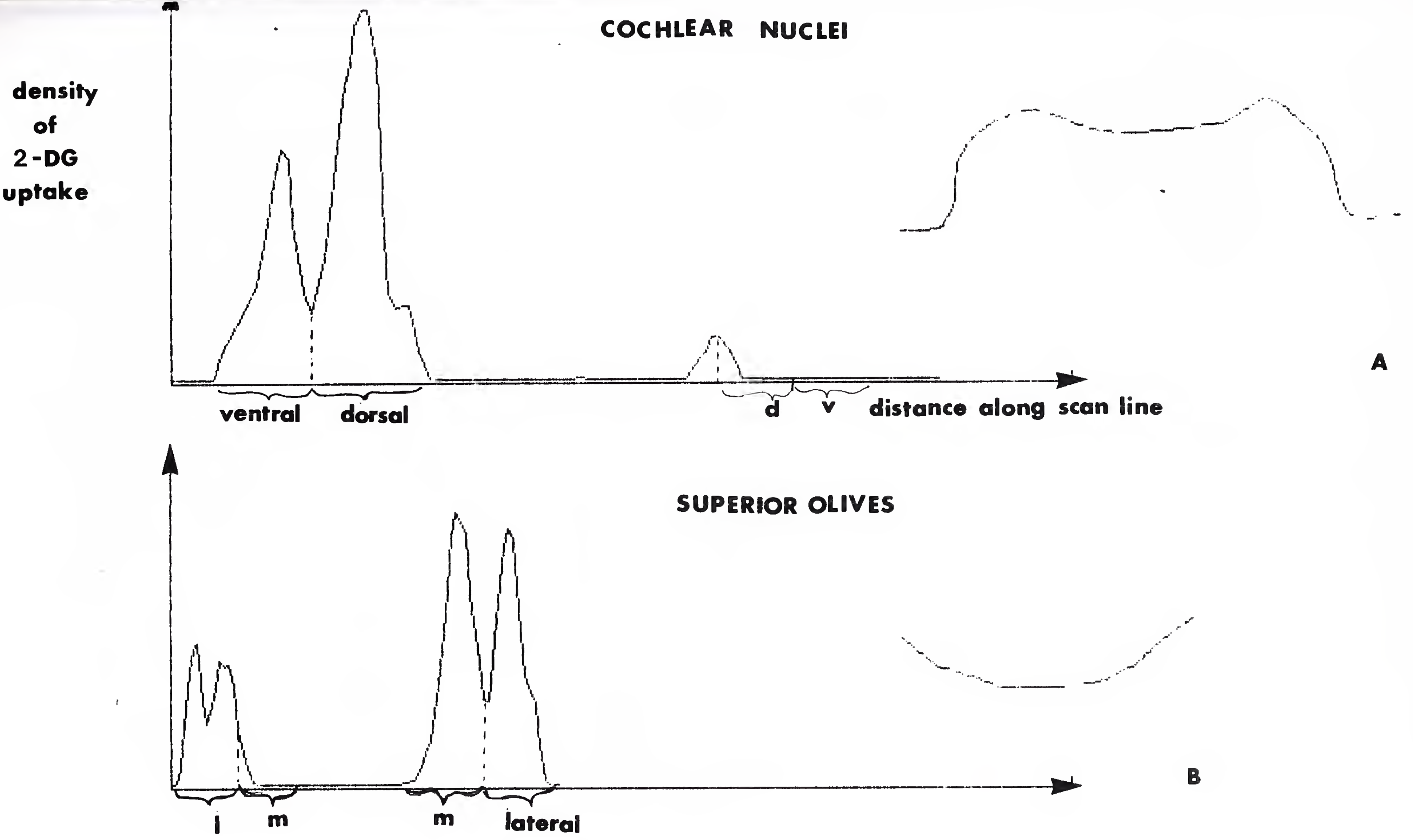


FIGURE 4.Computer-generated graphs plotting density of 2-DG uptake versus distance along a scan line for autoradiographs of brain sections from an animal sacrificed 2-3 hours after right cochlea blation. These plots were generated by moving the probe of a reflectance densitometer along a line crossing the auditory structures. A:level of the cochlear nuclei. B:level of the superior olivary nuclei.

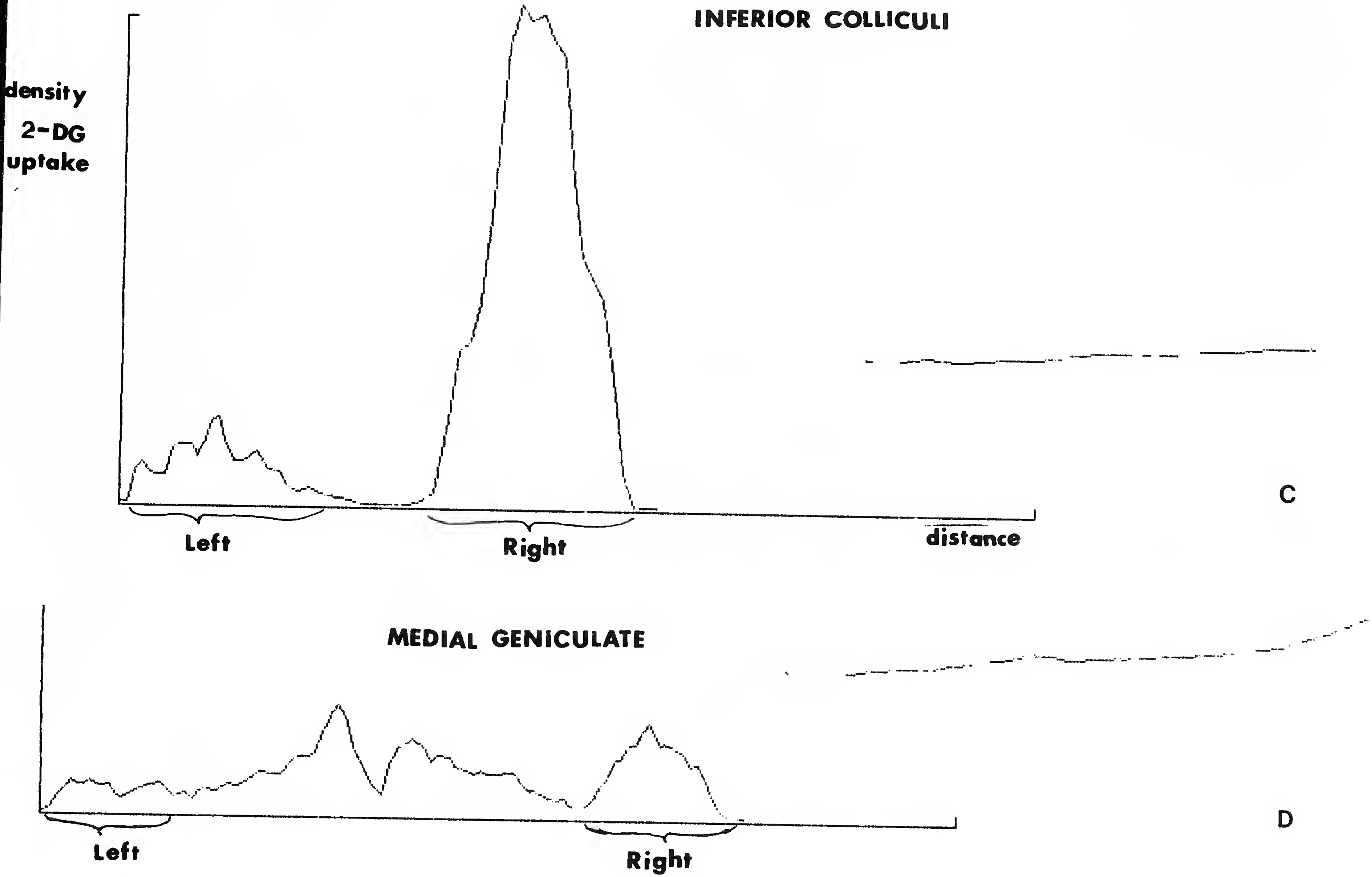


FIGURE 4. Continued. C:level of the inferior colliculi. D:level of the medial geniculate

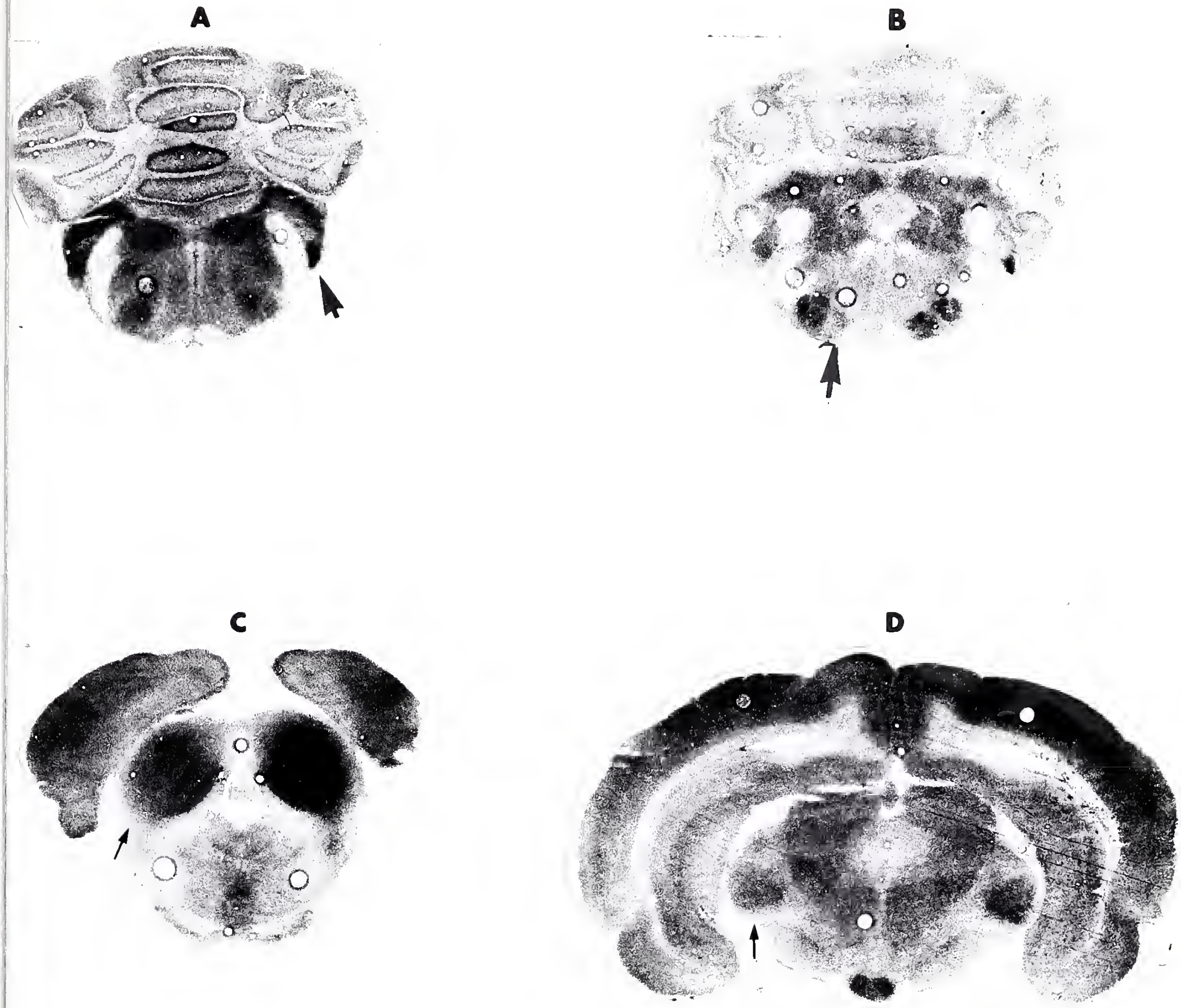


FIGURE 5. A: 2-Deoxyglucose autoradiograph of guinea pig brain at the level of the cochlear nuclei observed 42 days after right cochlea removal. B: section st the level of the superior olivary nuclei. C: section at the level of the inferior colliculi. D: section at the level of the medial geniculate bodies. In each photograph arrows indicate sites of reduced uptake compared to control.

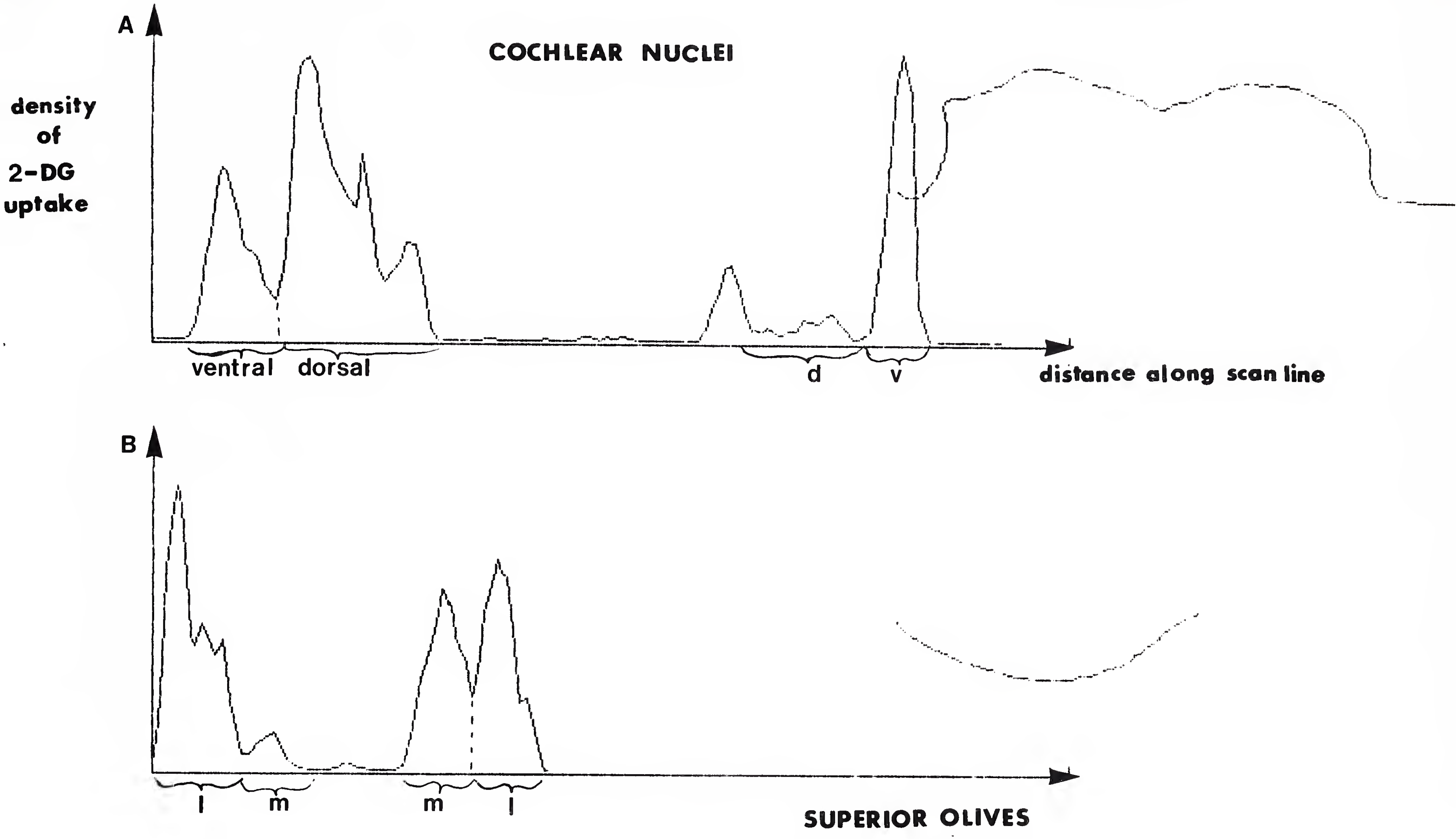


FIGURE 6. Computer-generated graphs plotting density of 2-DG uptake versus distance along a scan line for autoradiographs of brain sections from an animal sacrificed 42 days after right cochlea removal. These plots were generated by moving the probe of a reflectance densitometer along the line crossing the auditory structures. A:level of the cochlear nuclei. B: level of the superior olivary nuclei.

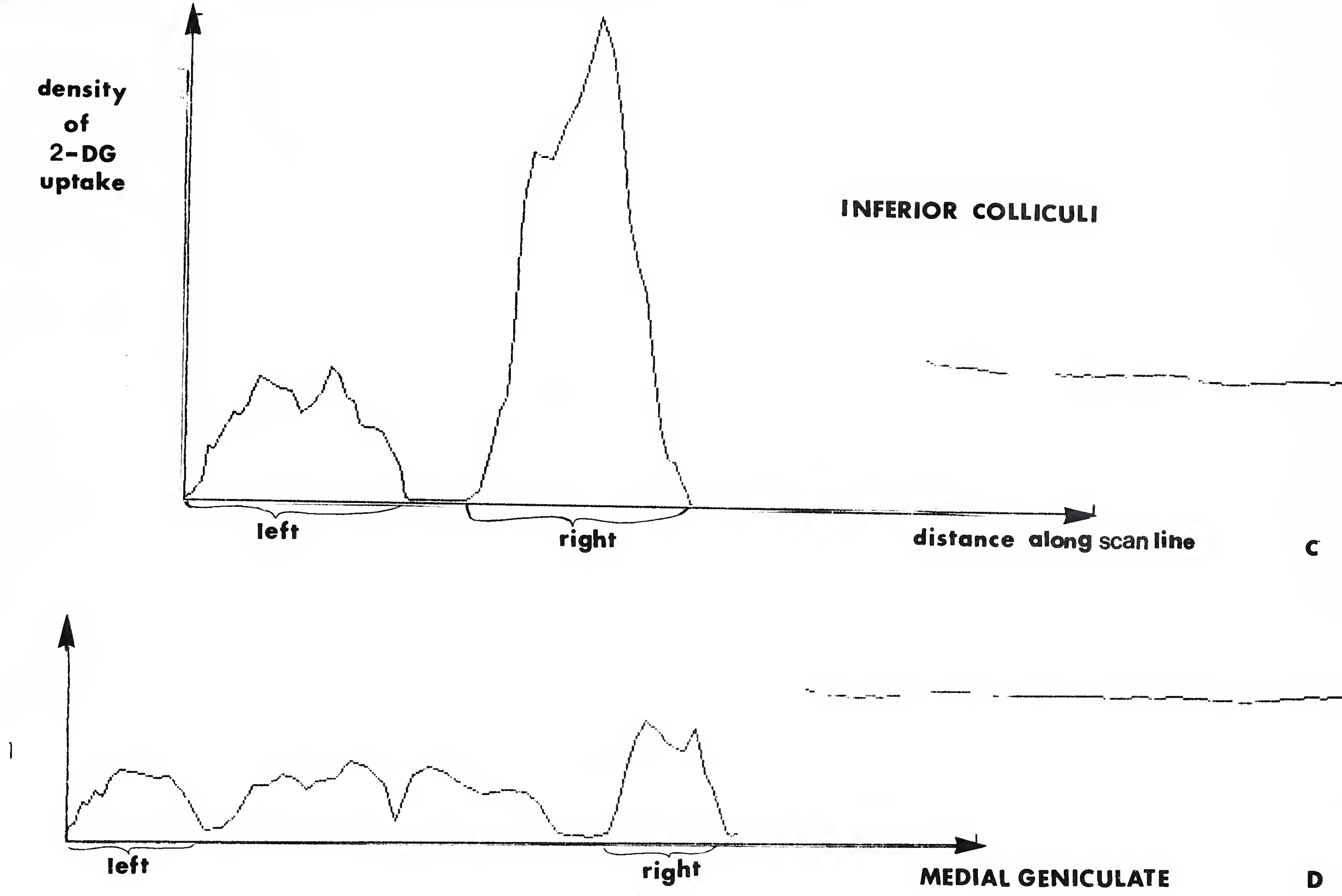


FIGURE 6 continued. C:level of the inferior colliculi. D: level of the medial geniculate bodies.

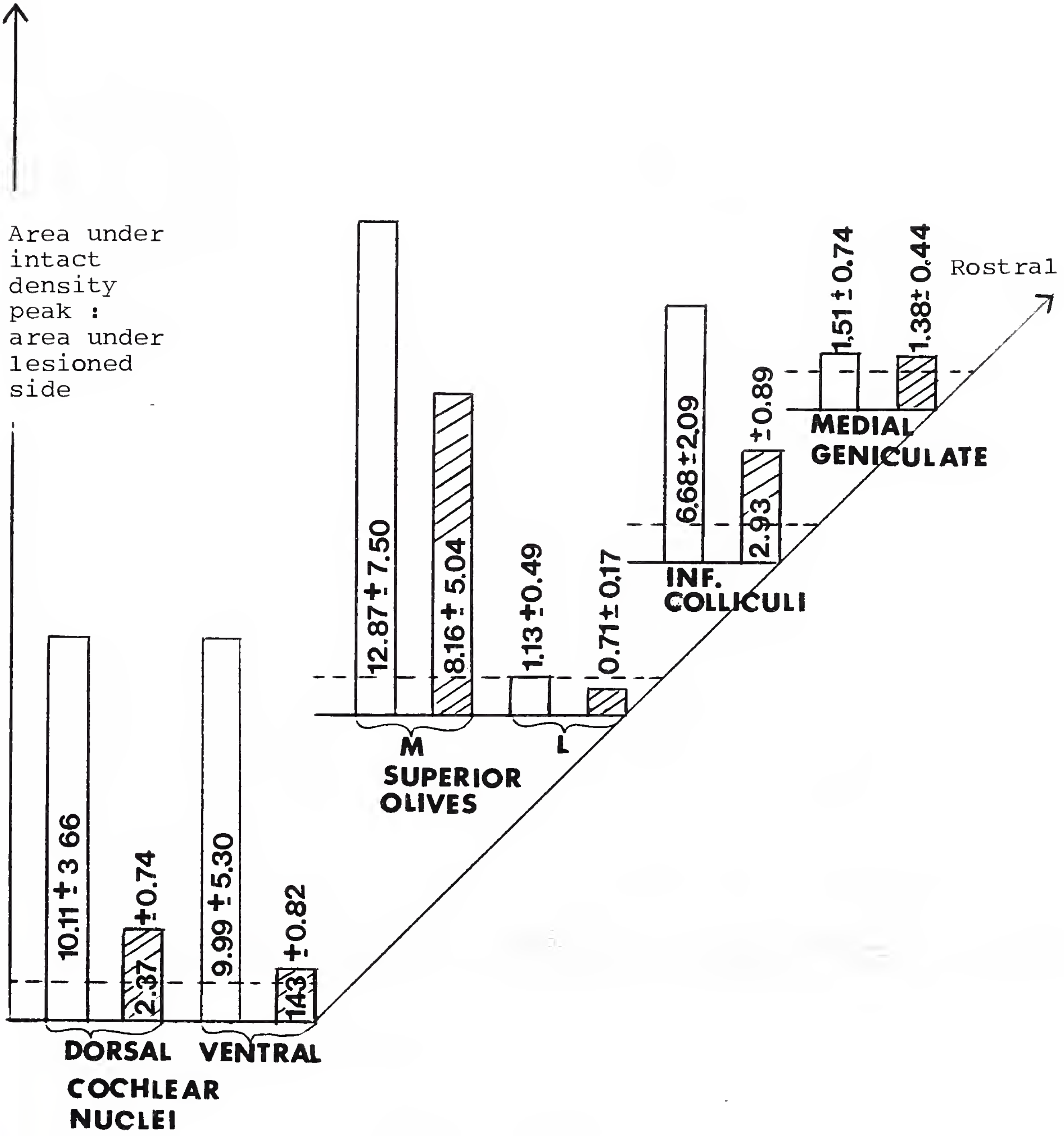


FIGURE 7. Graph comparing average area ratios (see text) for animals sacrificed several hours (acute) and 42 days after right cochlea removal. Acute animal indicated by clear bars, 42 animal indicated by cross-hatched bars. Broken line indicates ratio of 1 which approximates control. Numbers indicate mean area ratios ± standard deviation.

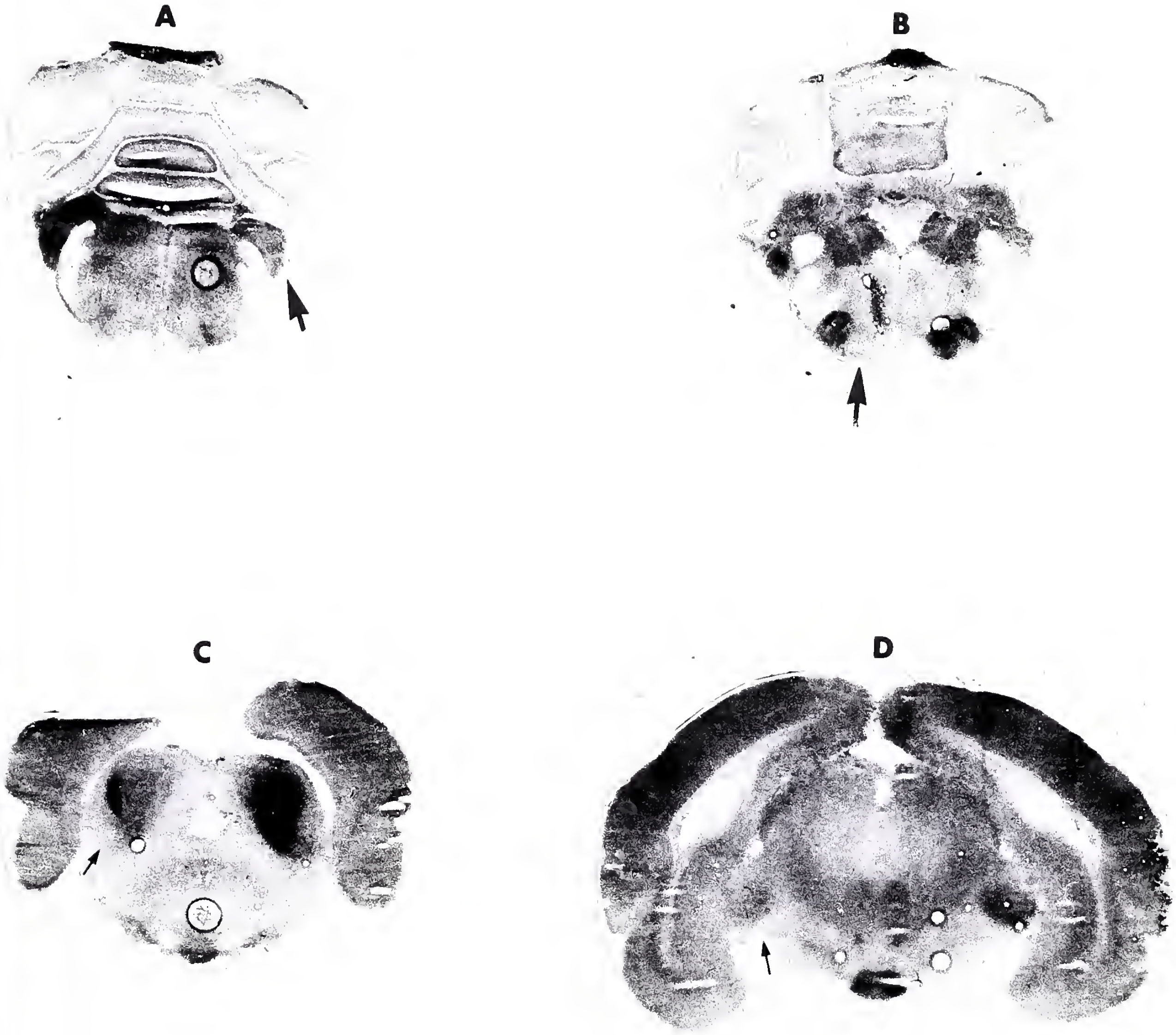


FIGURE 8. A: 2-Deoxyglucose autoradiograph of guinea pig brain at the level of the cochlear nuclei observed two days after right cochlea removal. B: section at the level of the superior olivary nuclei. C: section at the level of the inferior colliculi. D: section at the level of the medial geniculate bodies. In each photograph arrows indicate sites of reduced uptake compared to control.

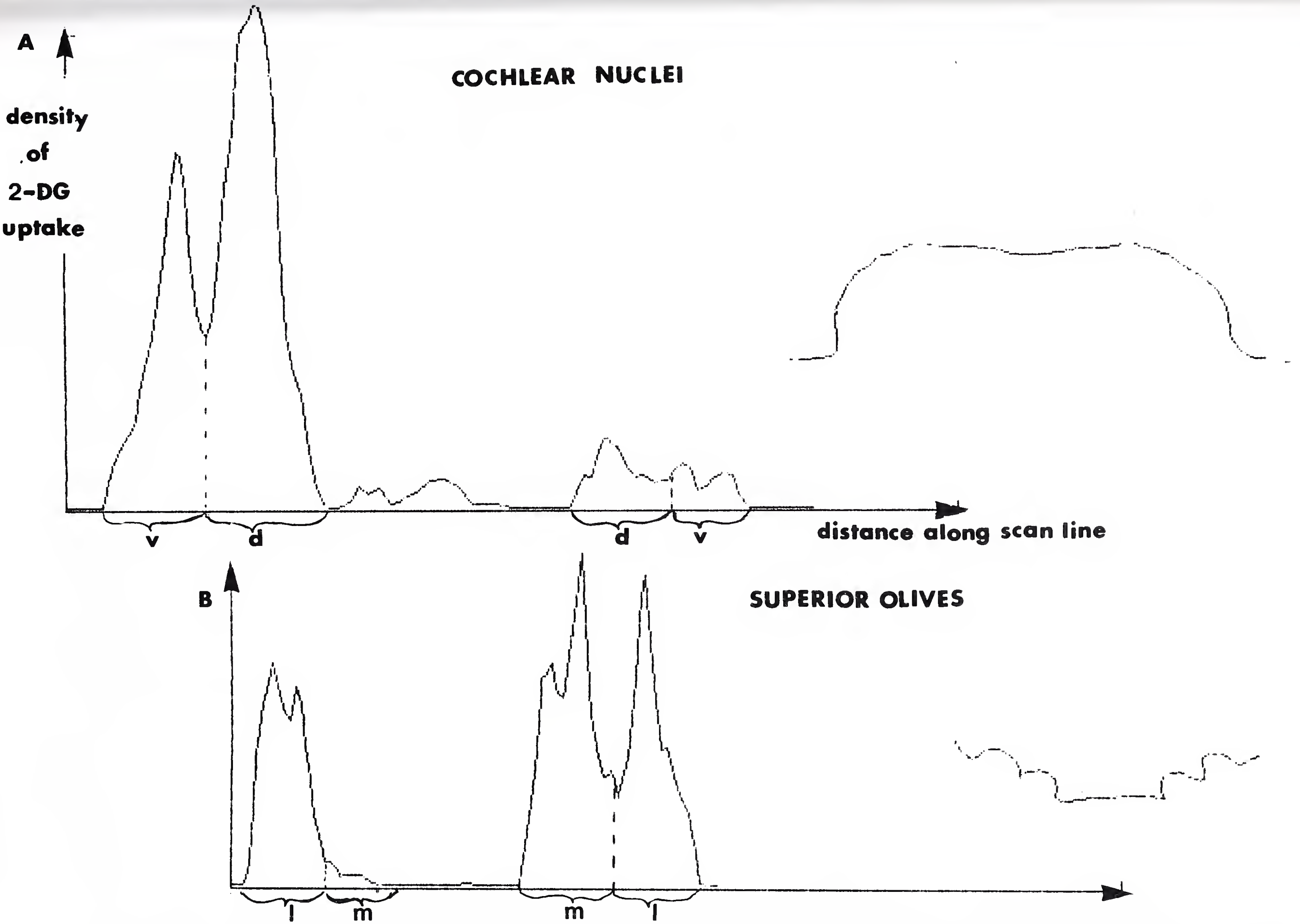


FIGURE 9. Computer generated graphs plotting density of 2-DG uptake versus distance along a scan line for autoradiographs of brain sections from an animal sacrificed two days after right cochlea removal. These plots were generated by moving the probe of a reflectance densitometer along the line crossing the auditory structures. A: level of the cochlear B:superior olives.

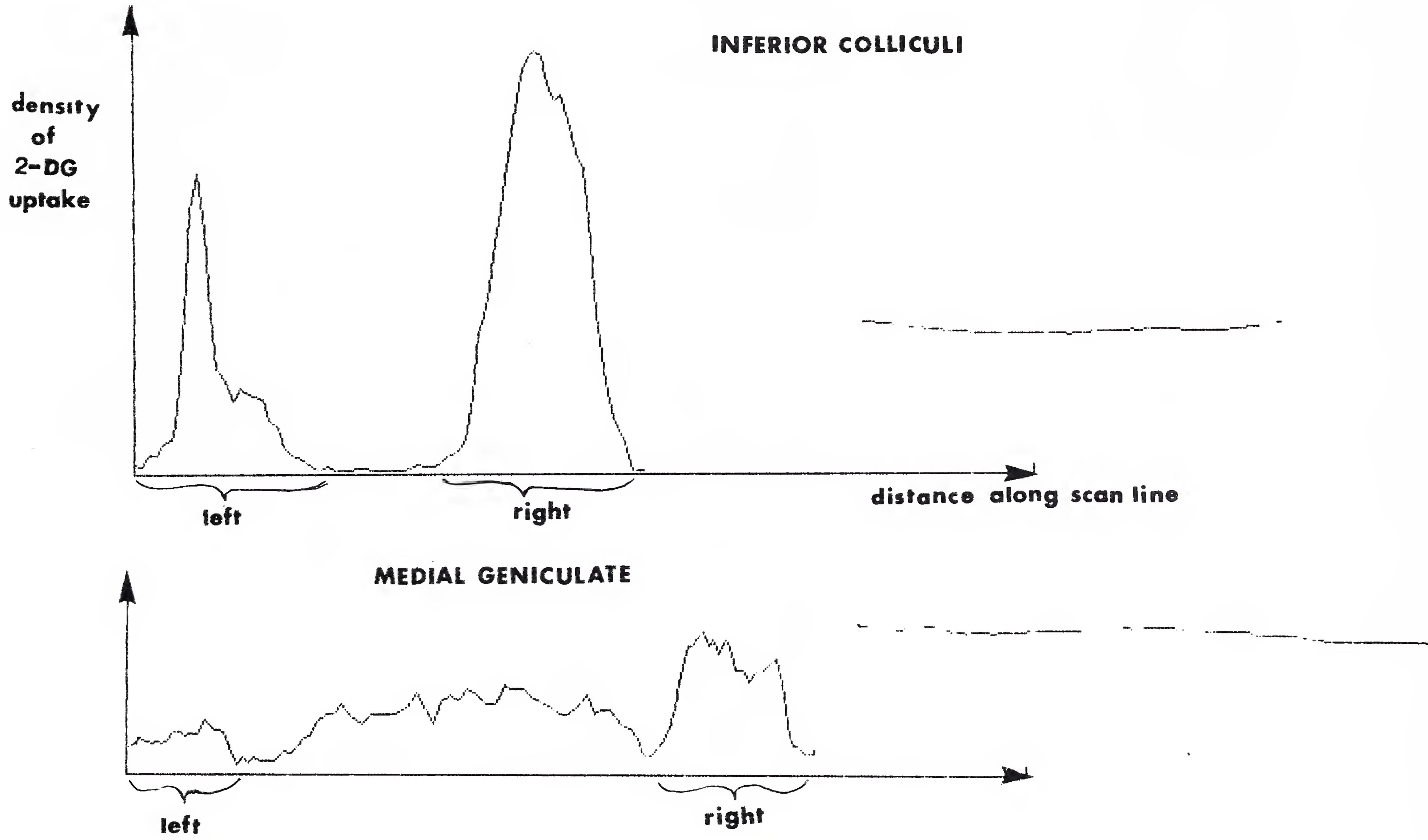


FIGURE 9 continued. C: level of the inferior colliculi. D: level of the medial geniculate

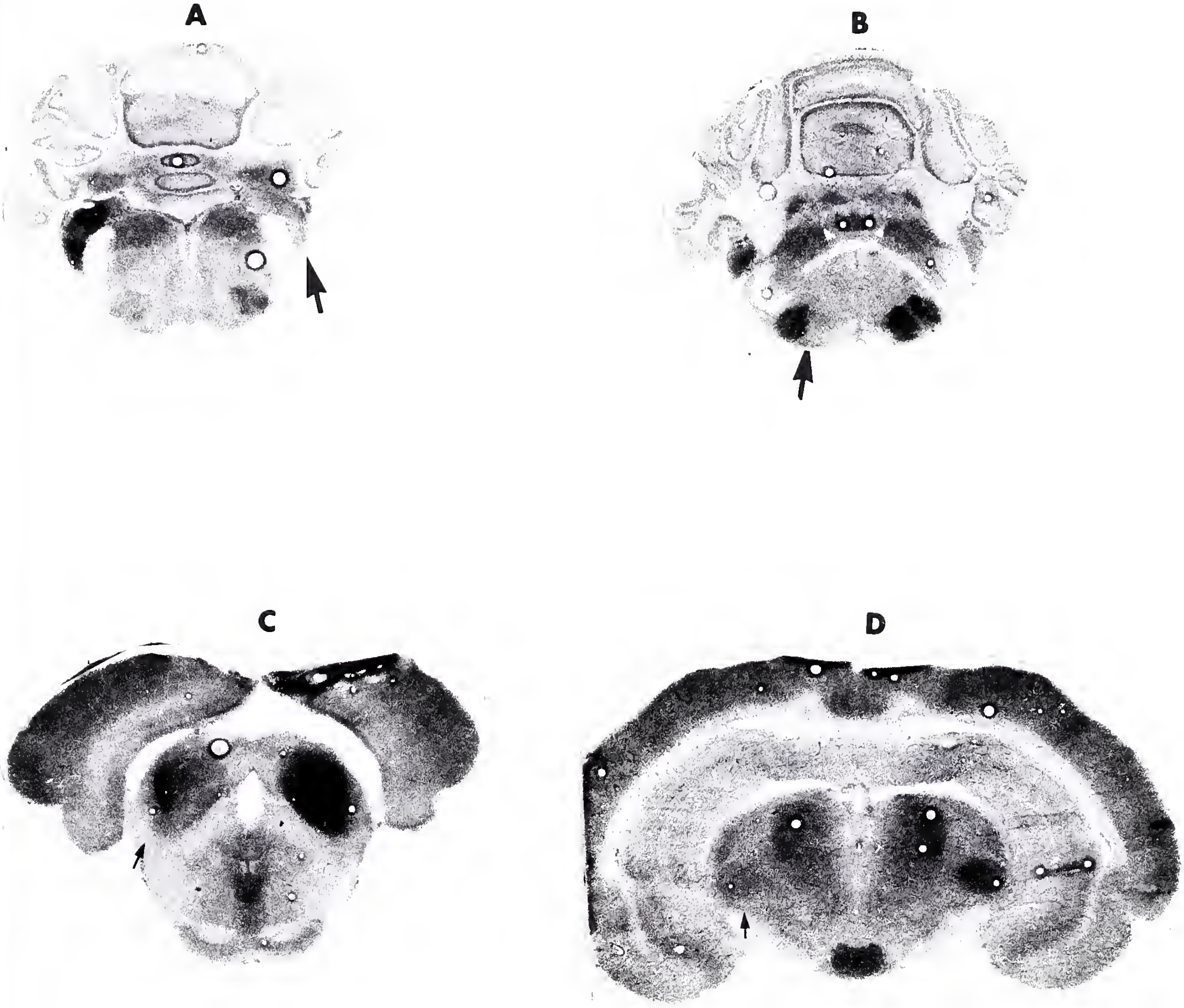


FIGURE 10. A: 2-Deoxyglucose autoradiograph of guinea pig brain at the level of the cochlear nuclei observed five days after right cochlea removal. B: section at the level of the superior olivary nuclei. C: section at the level of the inferior colliculi. D: section at the level of the medial geniculate bodies. In each photograph arrows indicate sites of reduced uptake compared to control.

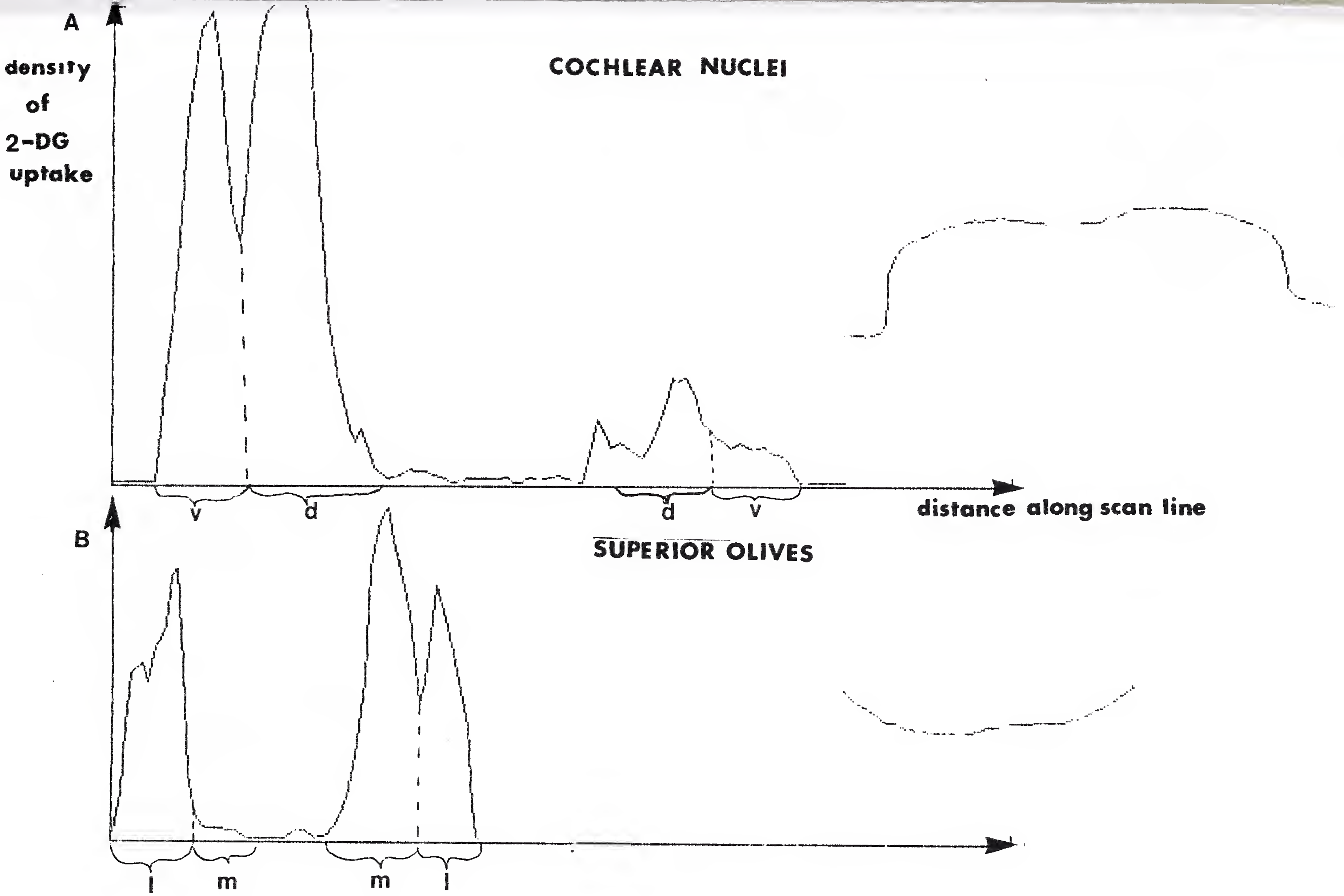


FIGURE 11. Computer-generated graphs plotting density of 2-DG uptake versus distance along a scan line for autoradiographs of brain sections from an animal sacrificed five days after right cochlea removal. These plots were generated by moving the probe of a reflectance densitometer along the line crossing the auditory structures. A:level of the cochlear nuclei. B:superior olives.

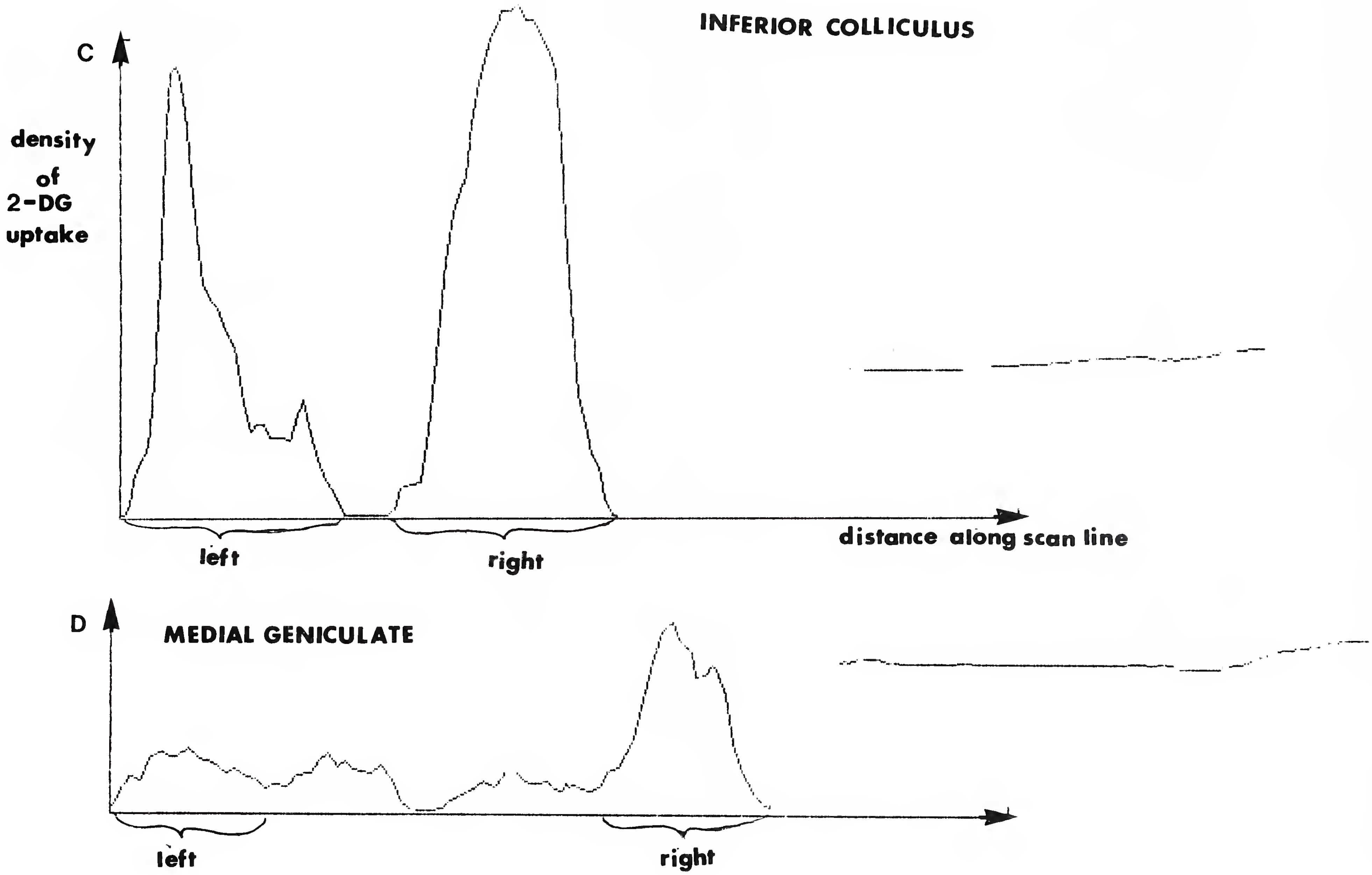


FIGURE 11 continued. C:level of the inferior colliculi. D:level of the medial geniculate

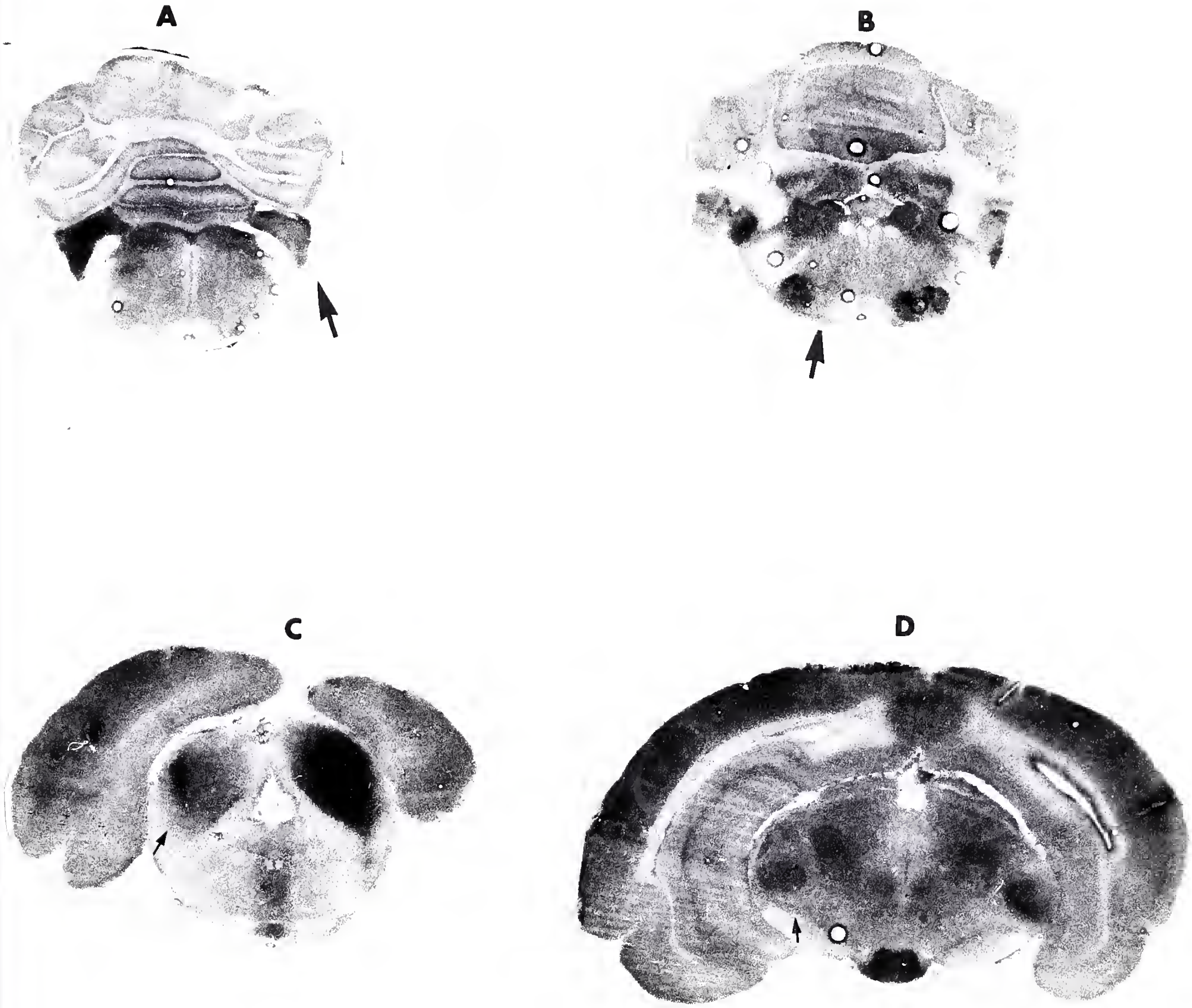


FIGURE 12. A: 2-Deoxyglucose autoradiograph of guinea pig brain at the level of the cochlear nuclei observed nine days after right cochlea removal. B: section at the level of the superior olivary nuclei. C: section at the level of the inferior colliculi. D: section at the level of the medial geniculate bodies. In each photograph arrows indicate sites of reduced uptake compared to control.

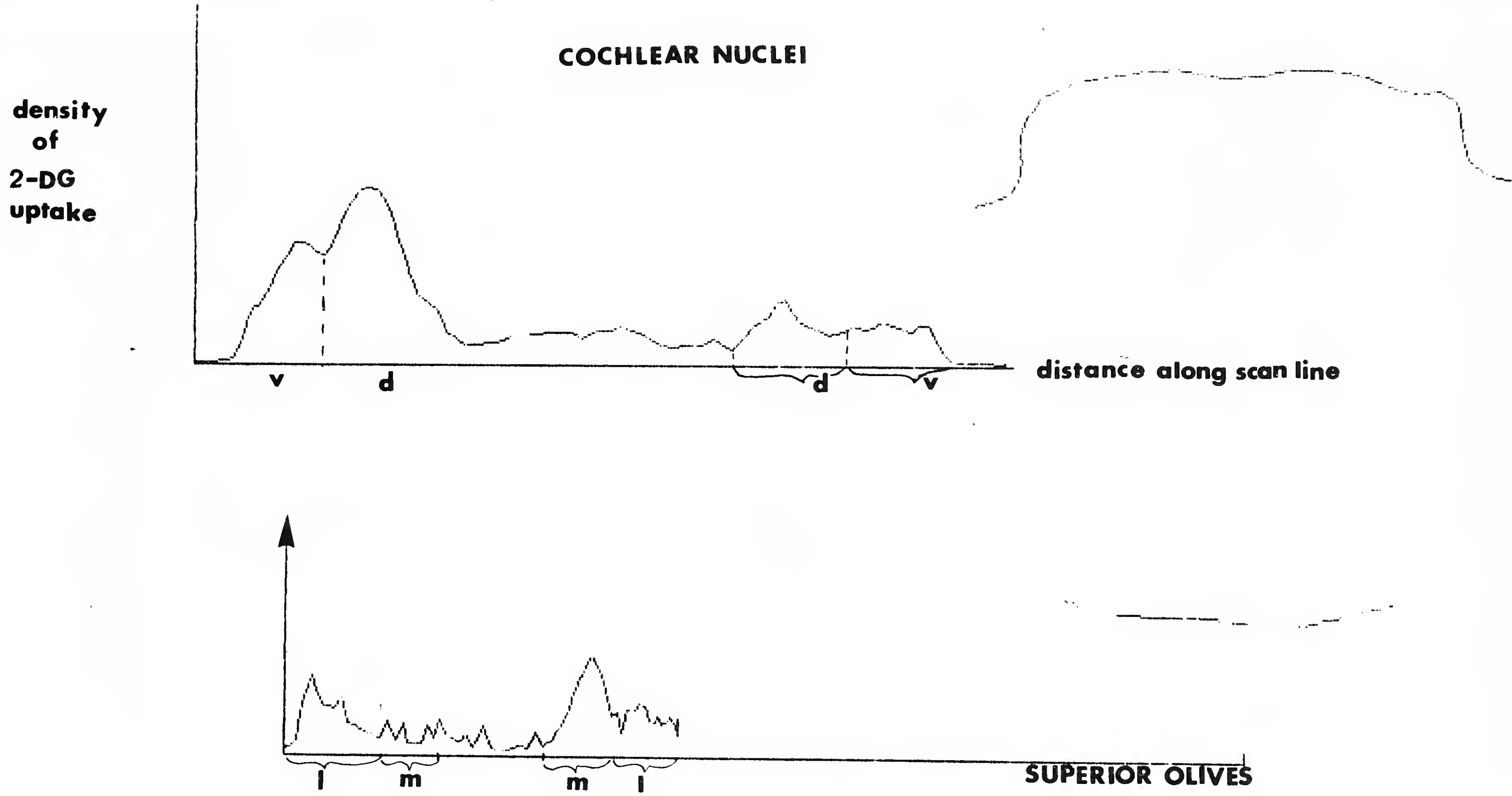


FIGURE 13. Computer-generated graphs plotting sensity of 2-DG uptake versus distance along a scan line for autoradiographs of brain sections from an animal sacrificed nine days after right cochlea removal. These plots were generated by moving the probe of a reflectance densitometer along a line crossing the auditory structures. A:level of the cochlear nuclei. B:Superior olives.

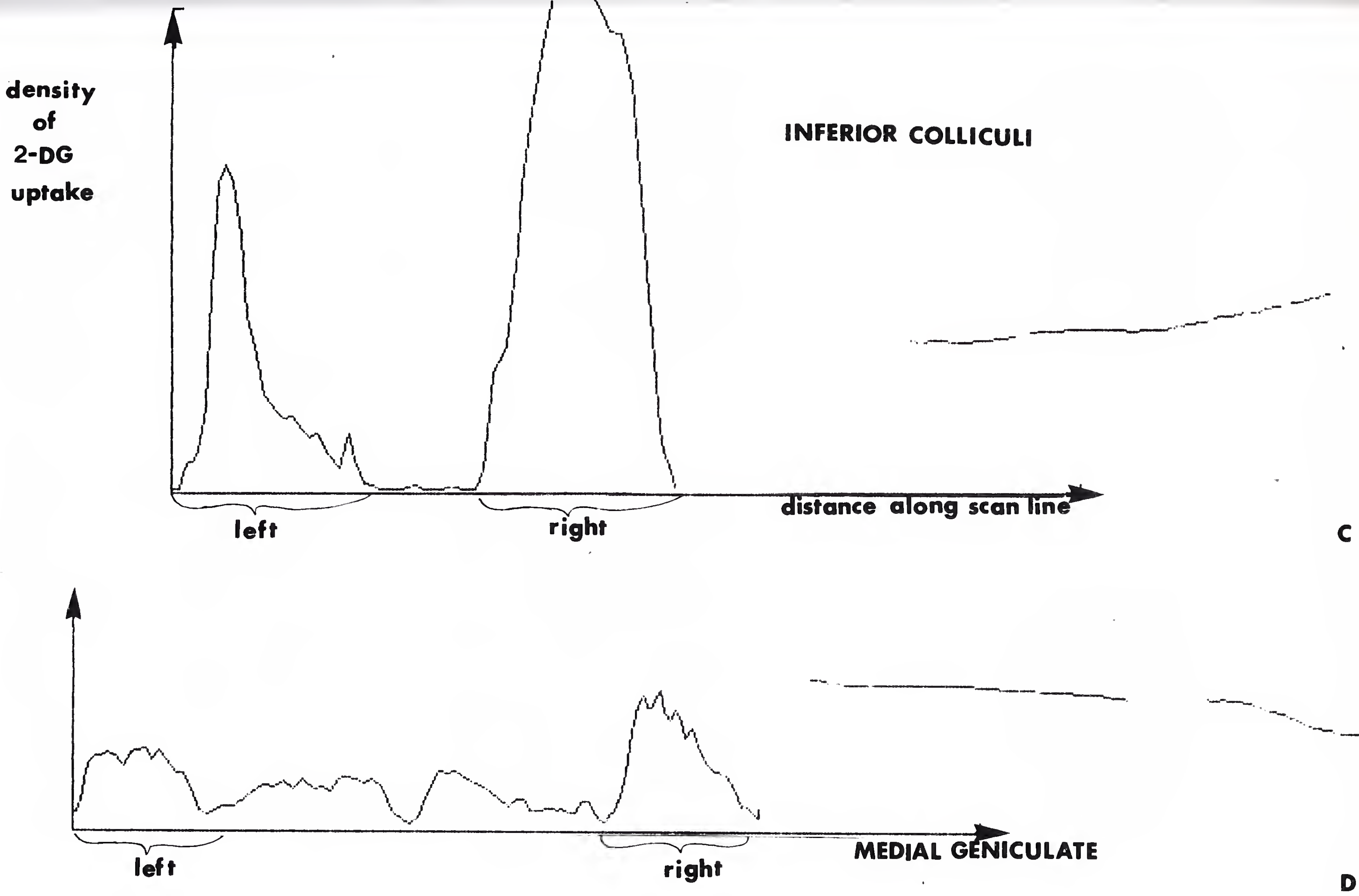


FIGURE 13 continued. C: level of the inferior colliculi. D: level of the medial geniculate bodies.

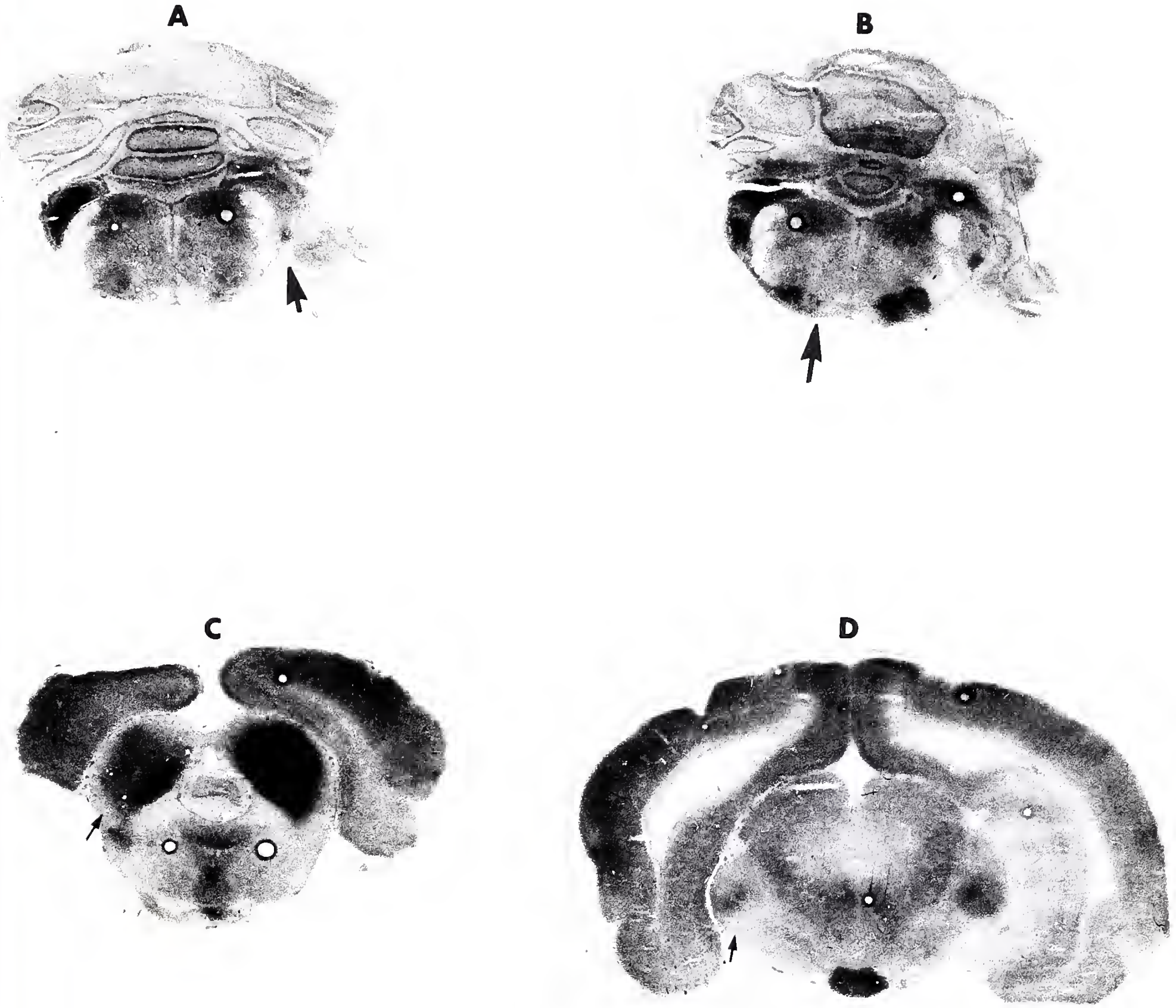


FIGURE 14. A: 2-Deoxyglucose autoradiograph of guinea pig brain at the level of the cochlear nuclei observed fourteen days after right cochlea removal. B: section at the level of the superior olives. C: section at the level of the inferior colliculi. D: section at the level of the medial geniculate bodies. In each photograph arrows indicate sites of reduced uptake compared to control.

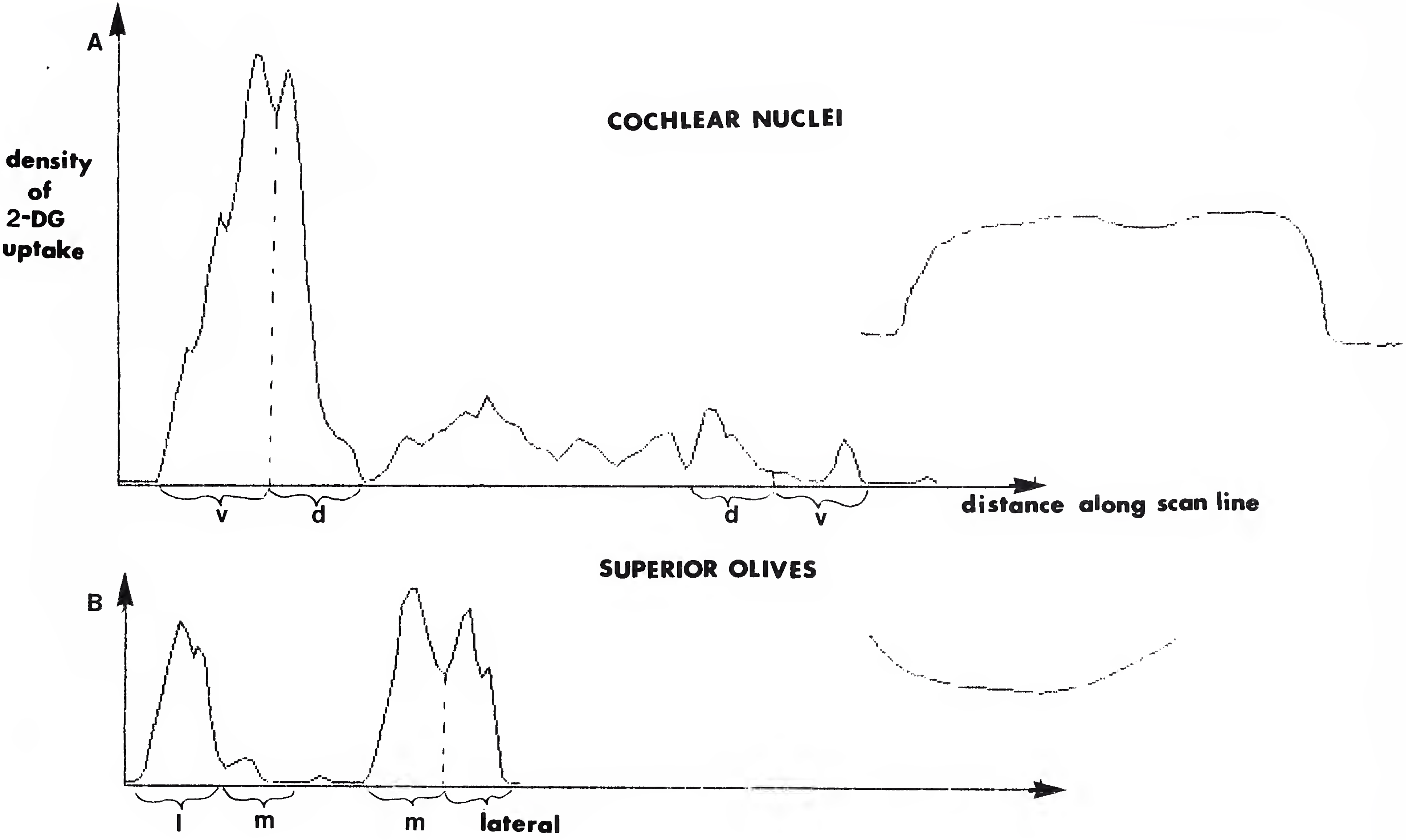


FIGURE 15.Computer -generated graphs plotting density of 2-DG uptake versus distance along a scan line for autoradiographs of brain sections from an animal sacrificed 14 days after right cochlea removal. These plots were generated by moving the probe of a reflectance densitometer along a line crossing the auditory structures. A:level of the cochlear nuclei. B:Superior Olives.

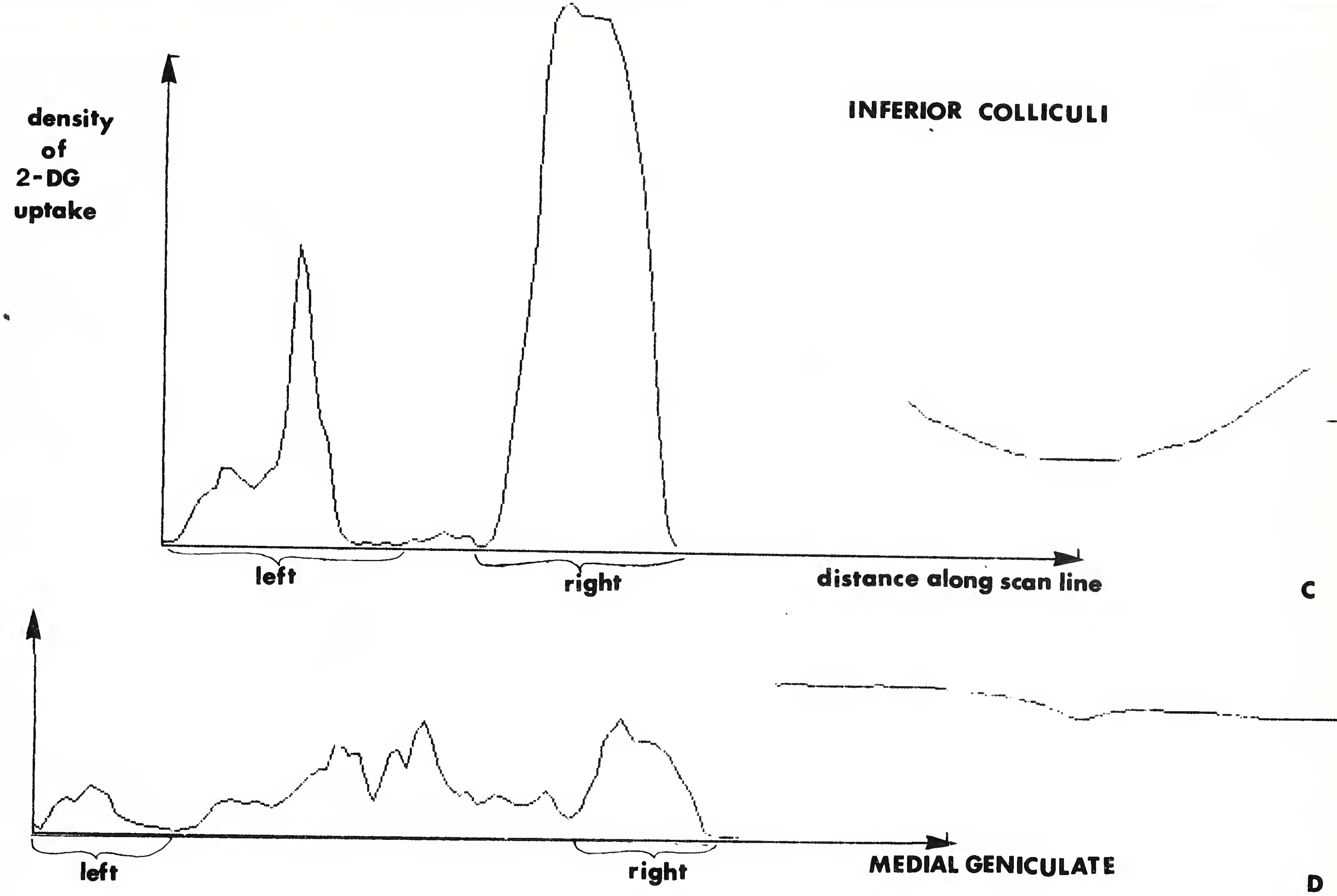


FIGURE 15 continued. C: level of the inferior colliculi. D: level of the medial geniculate bodies.

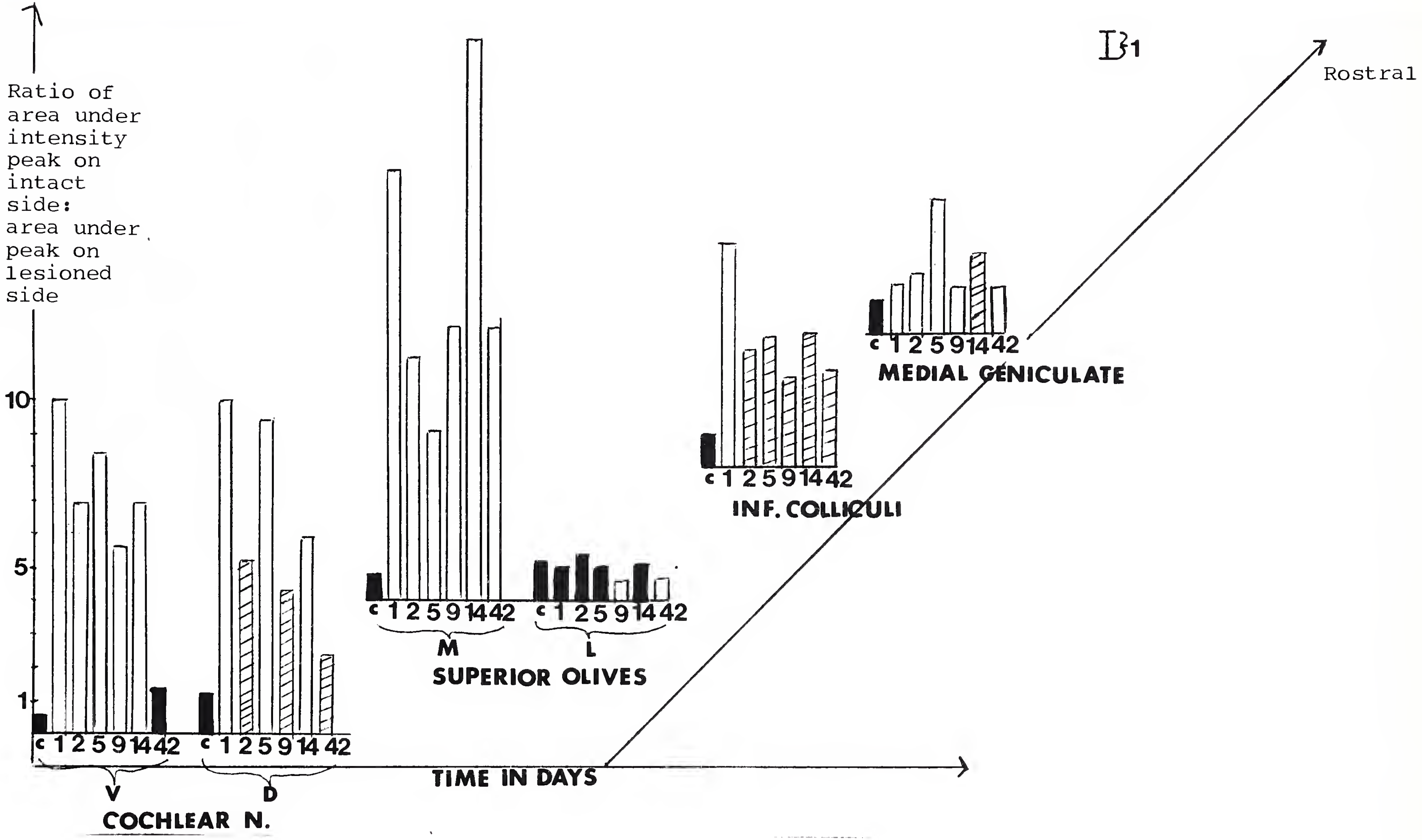


FIGURE 16. Summary graph of average area ratios (see text) versus time in days for each of the auditory structures examined. Values not significantly different than each other share the same pattern of shading. C refers to the control animal. Scale in upper right indicates five small boxes equals one unit.

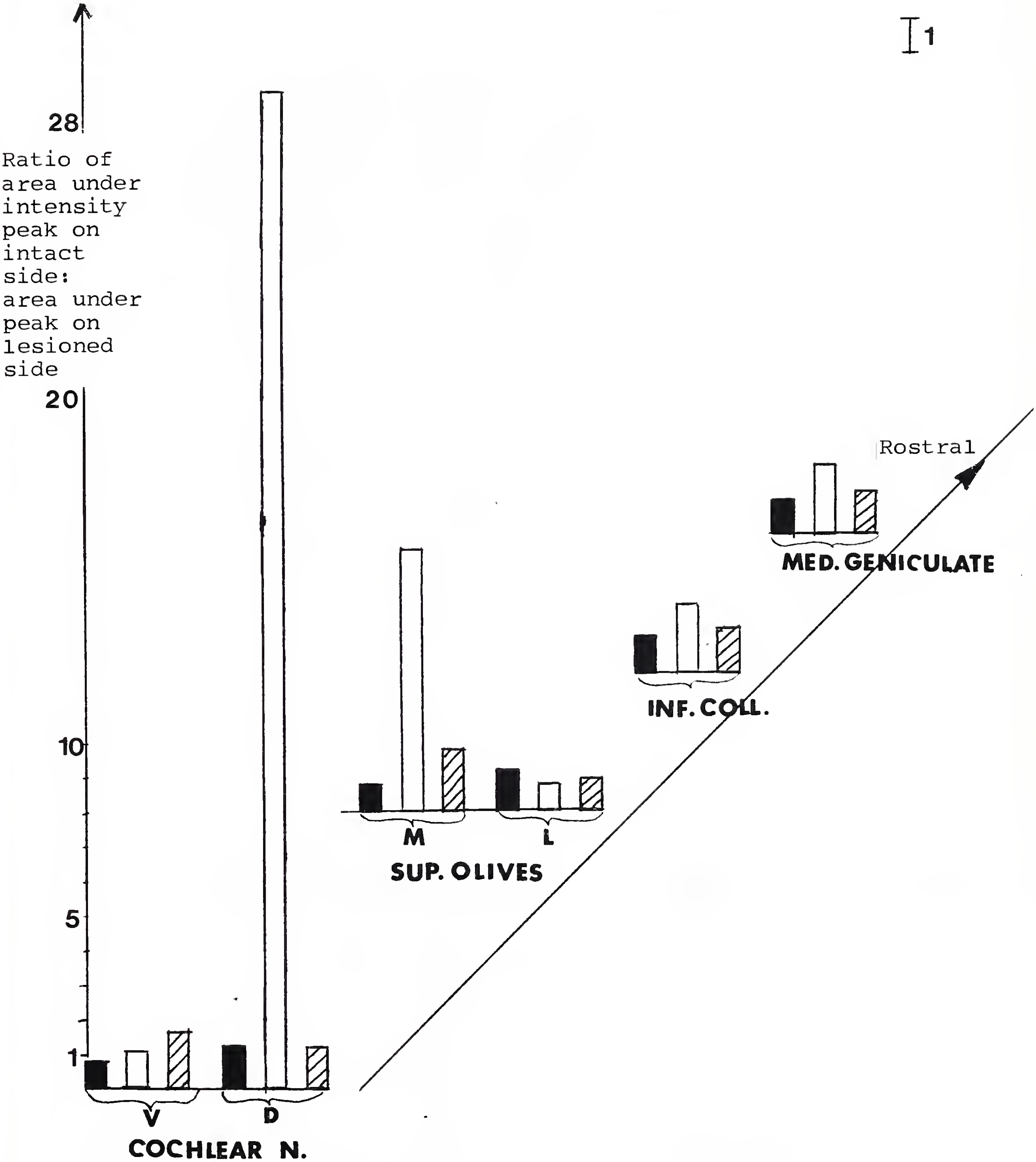
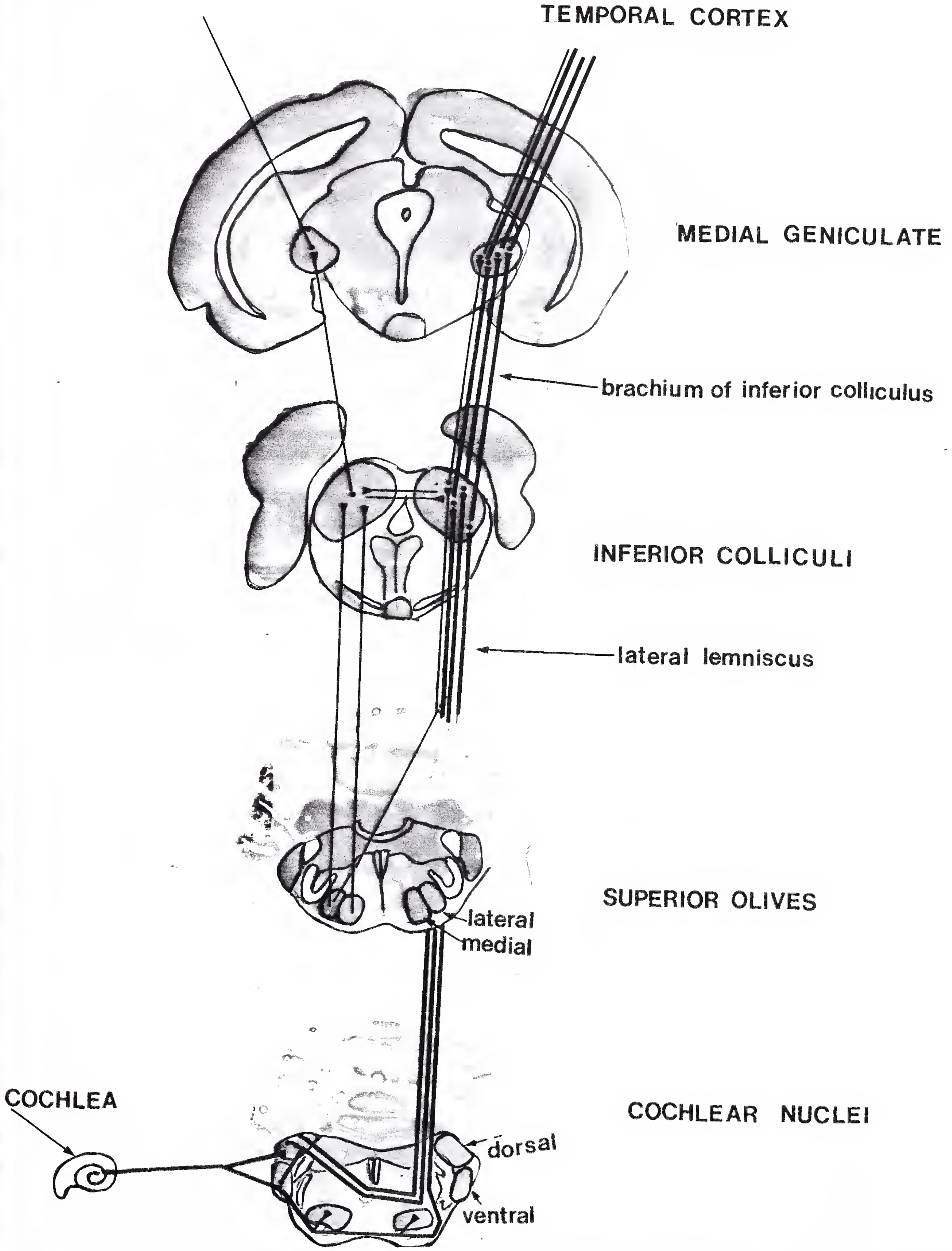


FIGURE 17. Graph comparing average area ratios (see text) for animals sacrificed several hours (acute) and 42 days after "sham-operation." Acute sham-op animal indicated by clear bars, 42 day animal indicated by cross-hatched bars, control animal indicated by solid bars.



GURE 18. Schematic representation of the ascending auditory pathways. Thick nes represent major pathways, thin lines represent quantitatively smaller thways. Modified from Carpenter, M.B. Human Neuroanatomy.7th ed.,Williams ilkens Company, 1976.

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